Food Research

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HEAT PROCESSING OF BEEF. IV. FUNCTIONAL RELATIONSHIPS OF TEMPERATURE, TIME, AND SPACE DURING PROCESSING AT HIGH RETORT TEMPERATURES[a]

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(Manuscript received July 28, 1954)

1. THE APPLICATION OF MATHEMATICS TO PRESERVING PROCESSES—A RECAPITULATION OF PRINCIPLES, OBJECTIVES, AND NOMENCLATURE

The necessity of application of the fundamental laws and practices of mathematics, bacteriology, engineering, physics, statistics and chemistry is becoming more and more evident to research workers investigating and developing preserving processes. Whereas in the past it was deemed sufficient to follow a few rules established by tradition and experience, at the present time a more fundamental approach is required to assure a product of highest possible quality and acceptability and at the same time one which is safe for consumption.

The place for application of the fundamental principles seems to be in the investigation of both the raw materials processed and of processing methods. It is difficult to differentiate between these two categories as methods depend on materials processed. It is possible, however, to group the raw materials in definite units according to their properties and develop general methods applicable to more than one commodity. Therein lies the paramount importance of fundamental research, the importance of investigation of fundamental laws which govern not one process but a whole domain of various phenomena which can be explained by the same basic principle applied to varying conditions. Once the fundamental laws are established, the prediction of the course of a process is facilitated without recourse to repeated experimentation, although additional data pertaining to the properties of materials processed and process conditions are required for prediction.

Mathematics furnishes means for expression of the fundamental laws and principles in a relatively short and precise manner whenever relationships of variable factors are involved. Moreover, seemingly unrelated physical phenomena can be expressed in the same general mathematical form.

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[a] Journal Paper J-2746 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1123.

[b] Formerly of Iowa State College, Ames, Iowa, where the experiments reported in this series were conducted.

Mathematical development of basic laws leads to the solution of specific problems for a specific set of conditions. The mathematical solution then can be verified by actual experimentation.

When pure (theoretical) mathematics fails or where theory cannot be applied readily to the problem in question, empirical relationships must be established from experimental data. The thinking process is reversed and instead of experimental verification of a postulated theoretical relationship, an empirical relationship is established from experimental data at hand. The empirical prediction equation may be derived in more than one way; a theoretical equation may be modified by including a correction factor or an equation of a different mathematical form may be deduced. Methods of establishing the equations differ widely with the field of research.

Statistical methods offer excellent tools in deriving prediction equations by means of least squares regression methods which give the best fitting curves once the form of functional relationship is postulated. Another advantage of statistical methods lies in the determination of experimental errors and sources of variation; these methods are of especial value in biological sciences where large variations are encountered in experimental materials. The use of statistical methods results in much better knowledge of precision in prediction as compared with visual curve fitting methods widely accepted in many fields of investigation. Where precision of determination is of primary importance the use of statistical methods is a practical requirement. Statistical design of an experiment facilitates greatly the interpretation of results and in most cases, saves labor and cost. However, in some cases a visual estimate is sufficient for practical purposes. It is up to the research worker to decide which tools to use. The degree of precision of experimental measurements should be a deciding factor. Unprecise measurements do not warrant derivation of equations representing the smallest detail of the experimental data as such prediction will not be precise. In engineering practice it is quite common to use the visual method of curve fitting; the relationships thus established have been found to be satisfactory for the practical purposes for which they were used. Visual curve fitting is also in common use in the field of food technology. It seems, however, that more rigorous methods of deriving of functions are generally of more value.

Experimental errors. Any investigation presents the problem of validity and precision of the results and should include consideration of the experimental errors. Experimental errors may be determined for the means of a number of observations and will be found to decrease as the size of the sample increases, thus the precision of the experimental results will usually be higher if more determinations are made. Another question arises in determination of experimental errors of results not directly measured but derived by mathematical formulae and depending on measurements of other variables; such errors are found to be dependent on errors in measurement of the component variables and from theoretical considerations one is able to estimate the precision of the result as a function of the partial errors in determination of these variables *(5)*. Standard statistical methods are used to estimate experimental errors connected with direct observations of various measurable properties. The estimate of the experimental error enables one to establish at defined probability level limits within which one

expects the results to be contained. These limits derived by rigorous methods indicate both the precision and reliability of the results.

In the past the consideration of experimental errors has been often disregarded in many fields of research; in other fields the considerations of experimental errors was not treated rigorously, often because there was no rigorous method available for such a treatment or because it was not considered important. However, any measurements even of homogeneous and "ideal" materials do involve variations from one observation to another, and when biological materials are tested an inherent variation within the material itself is added to the variations in measurement data. Therefore, results which do not contain estimates of experimental errors and their sources cannot be considered reliable and valid. An estimate of experimental errors and their sources should be included in the results of an investigation to give an idea of the precision of the experiment. In some cases, however, it might not be possible to estimate the errors by rigorous methods and only an approximate evaluation of errors is possible of attainment. The errors can be estimated from theoretical considerations of the propagation of precision indices *(5, 17)* when functional relationships are used to calculate results and standard statistical methods can be used if available for estimation of directly measurable properties.

Heat processing of meat. In the study of heat processing of meats as in all food preservation processes two objectives are to be attained: a palatable product acceptable to the consumer and a product which will not endanger the health or life of the consumer. These objectives have to be achieved simultaneously since attainment of one of them without the other renders the product unmarketable. To reach the above goals two general problems have to be considered: (a) the properties of the raw and processed material (physical, chemical and bacteriological); and (b) methodology of processing (specification of process in terms of length and processing temperature, and sterilizing value).

Three types of approach to such problems are conceivable:

(1) Purely practical approach based on past experience and tradition without any consideration for fundamental principles and laws behind the phenomena investigated.

This type of approach has been used in the past with varying degrees of success for various commodities. It has been quite successful in some cases, but often it has proved to be useless as far as achieving both of the above objectives. The development of the food industry and the increasing complexity of problems facing it has resulted in recognition of the importance of fundamental laws governing the heat processing of foodstuffs.

(2) Purely theoretical investigations based on idealizing assumptions and ideal materials. Very little consideration is given to the practical situation and highly hypothetical conditions are studied with little regard for the possible application to the solution of practical problems.

Upon cursory examination, this type of approach does not seem to be justified as it does not furnish answers to specific practical problems. In the long run, however, the understanding of fundamentals may prove absolutely necessary to provide a solution to complex problems. Over-emphasis on the theory may prove just as dangerous as disregard of it.

(3) A combination of the first two approaches involving an investigation of the fundamental laws governing the process with a view to the application of these fundamentals to practical conditions. An investigation is planned from the beginning to provide an answer to practical problems by means of ascertaining the basic fundamental principles involved, neither aspect being neglected. The precision of the results is taken into consideration by estimating the experimental errors which occur during the experimental phase of the research.

The investigation of canning processes represents a good example of application of fundamental principles to an actual manufacturing problem; it also serves as a good example of contributions from various branches of science to a problem in the field of food technology.

Review of previous investigations. A considerable amount of work on the fundamentals of heat processing has been done in the past. Little concern, however, has been shown for the evaluation of the precision of the results. A very meager amount of data available pertains specifically to meat and hardly any to beef. Under these circumstances, the applicability of general laws to processing of beef might be doubted. Actual experimentation and verification of the fundamental principles with the experimental data by means of rigorous estimate of the precision of results may decide whether the doubts have been justified. Experimental data are lacking for high-temperature process, as well as verification of cooling phase theory.

The small amount of information available in the literature concerning physical characteristics of meat and especially beef has been reviewed by Hurwicz *(9)* and by Bard *(3)*. Bard and Tischer *(4)* furnished additional data on objective evaluation of quality of beef connected with such physical characteristics as tenderness, moisture content, and fiber cohesiveness. Hurwicz and Tischer *(11)* discussed some theoretical considerations in heat processing of beef in cans in detail. The same authors *(12)* studied the temperature distributions in beef sealed in cans during thermal processing at two processing temperatures and determined the thermal diffusivity under these conditions. They discussed in detail experimental errors associated with the prediction equation derived and in various determinations made in the course of the experiment.

The situation is quite different in the field of evaluation of thermal processes necessary to assure commercial sterility of various commodities. A wealth of information is available in the literature pertaining to process determination and several methods have been worked out to ascertain the sterilizing value of thermal processes. Bard *(3)* and Hurwicz *(9)* reviewed some of these methods and investigations.

In the last decade a new approach to the problem of determination of an adequate process has been developed by Stumbo *(14, 15)*, Hicks *(7, 8)*, and Gillespy *(6)* and investigated by Hurwicz and Tischer *(12)*. The methods based on the new concepts involve a change in emphasis from the center location in the can to the whole can volume as a criterion of the adequacy of a process and the use of thermal death rate data for ascertaining the degree of sterility of cans rather than thermal death time data which suggests a total destruction of bacterial populations. To apply the newer concepts to the actual heat processing of various commodities it is neces-

sary to have detailed data on temperature distributions within cans packed with these commodities, and to derive the sterilizing value distributions from the temperature distributions. Such data are not available for actual canned foodstuffs except for the work of Hurwicz and Tischer *(12)*. Thus far in the case of some commodities the "safe" process resulted in an acceptable product, but in other cases and notably of beef, the "safe process" did not result in a canned product of high acceptability.

Objective evaluation of quality. Whereas the fundamental laws of heat transfer and thermobacteriology were applied to the problem of process evaluation, no fundamental laws can be found applying to the problem of objective evaluation of the quality of meat in terms of its tenderness and juiciness. No functional relationships were postulated relating the conditions of thermal process (processing temperature, time of processing) with the resulting shear force of the product or the drained juice and the lethality of the process. Very few references are available correlating even any two of the above factors *(4)*. A need for establishing such functional relationships as an introduction to establishment of general fundamental laws seemed to be urgent. Part III *(16)* of this series described an investigation intended to fulfill such needs.

A continuous failure to arrive simultaneously at a commercially sterile, palatable product when processes at relatively low temperatures (212-250° F) are employed leads one to believe that perhaps a high-temperature short-time process would ultimately produce a better product. No information was found available in the literature pertaining to the high-temperature process applied to meat products.

Very few investigations *(7, 12, 13)* concerned themselves with evaluation of experimental errors encountered in food research.

It seems then that a serious need exists to enrich and develop the present data on thermal and other physical characteristics of meat, to develop further the application of fundamental principles of heat conduction to the heat processing of beef during all phases of the thermal process, to correlate the physical changes occurring during heat processing with the lethality requirements and thus to obtain a new method of process determination for a safe and palatable product, and finally to compare the methods of visual curve fitting and those of regression techniques in the light of experimental errors. Exploration of a wide range of processing temperatures seems to be of especial value. An investigation of the bacterial distributions within the container would help greatly in testing the volume lethality concepts; this last investigation would, however, be beyond the scope of this study.

Objectives of the Investigation

In the light of the preceding discussion, the objectives of this investigation were set up as follows:

1. To determine some thermal properties of beef (thermal diffusivity, characteristics of heating and cooling curves) and temperature distribution during processing.
2. To conduct the investigation at a wide range of temperatures to include and to emphasize the high retort temperatures close to 300° F.

3. To investigate heating and cooling process with the emphasis on the phase starting after the steam is shut off and cooling water started.
4. To obtain empirical relationships among variables encountered in heat processing and to determine and correlate the physical changes in beef with process requirements for a sterile and acceptable product.
5. To compare the visual method of curve fitting with the statistical regression method of determination of slopes of heating and cooling curves.

The general purpose was to establish wherever possible, the fundamental laws expressed in mathematical form, or to arrive at mathematical empirical functional relationships embracing as large a realm of conditions as possible. This is to be achieved by statistical methods of evaluation and with emphasis on experimental errors associated with derived expressions.

The authors in the published three parts of this series attempted to follow the above established objectives. Part I *(11)* serves as an introductory paper explaining in detail the fundamental laws of heat conduction and deriving step-by-step the heat conduction equation commonly used in food technology applications. In Part II *(12)* the authors investigated thermal properties of beef canned in No. 2 cans, arriving at empirical time-temperature-space relationships during the heating phase of processing by the use of statistical methods. The experimental errors were determined and discussed. The relationship between the theoretical predictions resulting from the fundamental laws and the experimental results was ascertained. In Part III *(16)* the investigation was extended to higher than conventional temperatures up to 315° F., and the results of the objective evaluation of the quality were reported by means of mathematical correlation of the quality attributes with other process variables (time and temperatures). Again, attempts were made whenever possible on establishment of fundamental laws governing such functional relationships.

Part IV of the series, presented in this paper, is an investigation of the fundamental laws governing the thermal process of beef extended to high temperatures in 300 x 308 cans. Empirical functional time-temperature-space relationships are obtained for both cooling and heating phase for an extremely large number of determinations in the whole space of the container. The equations resulting from this investigation are obtained by rigorous statistical methods and carry an experimental error estimate. In the authors' opinion, the information contained therein as in the papers to follow, contributes to the knowledge of beef properties specifically as well as to the knowledge of high temperature processes. The information is obtained by rigorous statistical methods and thus is believed to be reliable, eliminating personal bias from the evaluation. The next paper in this series will cover the temperature distributions in the processed cans with an analysis of their occurrence. (Part V). Thermal diffusivity and "slopes" of heating and cooling curves (f_h and f_{ct}) and their determinations will be discussed in the following paper of the series. The intermediate cooling phase starting immediately after the steam shut-off will be the topic of another paper; and finally the sterilizing effect investigation will be reported in the last paper of the series. All these investigations were carried out for high-temperature processes.

NOMENCLATURE

a — radius of the container — (cm).

$a_{11} = A_{11}J_0(\mu_1 r)$ sin $\lambda_1(z+l)$ — intercept coefficient of the heating curve Eq. 2c, in first approximation of the theoretical equation, in semi-log plot; or the antilog of the intercept of log $[(RT-CT)/(RT-IT)]$ axis plotted vs t when $t=0$ — (dimensionless).

$\hat{a}_{11}$ — intercept coefficient determined from regression calculations of the heating curves from experimental data.

$\hat{a}'_{11}$ — intercept coefficient determined from Eq. 6a using calculated $\hat{j}$ values from cooling curves.

$b' = +k(\mu_1^2+\lambda_1^2)$ — slope of the heating curve on semi-log — (min.$^{-1}$).

$b_h = b' \log e$ — same as above for logarithm to base 10.

$\hat{b}_h$ — same as above determined by regression calculations from experimental data.

b'' — slope of the cooling curve as defined in Eq. 5a for semi-log plot (min.$^{-1}$).

$b_{cl} = b'' \log e$ — same as above for logarithm to base 10.

$\hat{b}_{cl}$ — same as above determined by regression calculations from experimental data.

f_h — "slope" of the heating curve as commonly used, actually reciprocal of $-b_h$ — (min.).

f_{cl} — same as above for cooling curves.

$g = (RT-CT)_u$ — temperature difference between the retort and can at the end of the heating and beginning of cooling — (°F.).

j — intercept coefficient of the cooling curve Eq. 5c, or the antilog of the intercept of log $[(CT-TC)/(CT_u-TC)]$ axis plotted vs $(t-t_l)$ when $(t-t_l)=0$ for all locations in the can. Also defined in Eqs. 5a and 6a (dimensionless).

$\hat{j}$ — intercept coefficient of the cooling curve determined by regression calculations from experimental data.

$\hat{j}_1$ — intercept coefficient of the cooling curve determined from Eq. 6a using $\hat{a}_{11}$ values.

k — thermal diffusivity — (cm^2 × min.$^{-1}$).

$2l$ — height of the can — (cm).

$m = (CT-TC)$ — temperature difference between the can and the cooling water — (°F.).

r — cylindrical coordinate (distance from central vertical cross-section of the can) — (cm).

t — time — (min.).

t_l — time when heating ends and cooling begins — (min.).

z — cylindrical coordinate (distance from central horizontal cross-section of the can) — (cm).

$x_{cl} = (t-t_l)$ — (min.).

$A_{im} = -4[(-1)^m-1]/m\pi(\mu_i a)J_1(\mu_i a) = 4(1-\cos m\pi)/m\pi(\mu_i a)J_1(\mu_i a)$ — constant — (dimensionless).

$C_1 = \hat{a}_{11}/a_{11}$ — heating lag coefficient (based on heating data) — (dimensionless).

$C_2 = \hat{a}'_{11}/\hat{a}_{11}$ — cooling lag coefficient (based on heating data) — (dimensionless).

$C_3 = \hat{j}/j$ — cooling lag coefficient — (dimensionless).

C.V. — coefficient of variation — (%).

CT — can temperature at a location — (°F.).

IT — initial temperature throughout the can — (°F.).

RT — retort (or processing) temperature — (°F.).

TC — cooling bath temperature — (°F.).

$J_0(\mu_i r)$ — Bessel function of first kind, zero order.

$J_1(\mu_i a)$ — Bessel function of first kind, first order.

$(\mu_i a)$ — positive zeros infinite in number $(i=1, \ldots, \infty)$ of $J_0(\mu_i a)=0$.

μ_i — positive roots infinite in number $(i=1, \ldots, \infty)$ of $J_0(\mu_i a)=0$.

$\lambda_m = \dfrac{m\pi}{2l}$ — constant depending on can size — (dimensionless).

Subscripts and other symbols

i — numbers $1, \ldots, \infty$ denoting the number of terms in series Eq. 1.

m — numbers $1, \ldots, \infty$ denoting the number of terms in series Eq. 1.

h — denotes values pertaining to the heating phase, or determined during that phase.

cl — denotes values pertaining to the cooling phase, or determined during that phase.

t_l — denotes values of variables at time $-t_l$.

$\hat{}$ — caret — used to denote experimental values determined by regression calculations.

2 FUNCTIONAL RELATIONSHIPS OF TEMPERATURE, TIME, AND SPACE DURING PROCESSING AT HIGH RETORT TEMPERATURES

The thermal characteristics of canned product such as thermal diffusivity of material, patterns of heat penetration and temperature distributions during heat processing with all their secondary effects exert considerable influence on the ultimate quality of the product. These characteristics depend on several variables the importance of which was investigated during the course of this experiment. The assumptions made during the investigation were discussed in Part I and II of this series *(11, 12)* and are the same as in *(11)*. The theoretical relationship referred to in several instances below is reproduced here as follows:

$$CT = RT - (RT - IT) \sum_{i=1}^{\infty} \sum_{m=1}^{\infty} A_{im} J_0(\mu_i r) \sin \lambda_m (z + l)\, e^{-k(\mu_i^2 + \lambda_m^2)t} + \qquad \text{Eq. 1}$$

$$\begin{cases} 0 & \text{for } 0 < t < t_1 \\ (TC - RT)\left[1 - \sum_{i=1}^{\infty} \sum_{m=1}^{\infty} A_{im} J_0(\mu_i r) \sin \lambda_m (z+l) e^{-k(\mu_i^2 + \lambda_m^2)(t - t_1)}\right] & \text{for } t > t_1 \end{cases}$$

where CT is the can temperature at a location; RT is the retort temperature (or processing temperature); IT is the initial temperature throughout the can; and TC is the cooling bath temperature; and temperatures can be measured either in °F. or °C. as long as consistent. A_{im} is a constant depending on the initial temperature distribution in the can assumed to be uniform.

$$A_{im} = \frac{-4\left[(-1)^m - 1\right]}{m\pi\,(\mu_i a)\, J_1(\mu_i a)} = \frac{4\,(1 - \cos m\pi)}{m\pi\,(\mu_i a)\, J_1(\mu_i a)}$$

$J_0(\mu_i r)$ is the Bessel function of first kind, zero order which can be found in tables of Bessel cylindrical functions, and which is equal to 1 in the center at $r = 0$.

$J_1(\mu_i a)$ is the Bessel function of first kind, first order and

$(\mu_i a)$ = positive zeros infinite in number of $J_0(\mu_i a)$ = or

μ_i = positive roots infinite in number of $J_0(\mu_i a)$ =

all of which can be found in tables of Bessel functions, and t is time; t_1 is time when heating is terminated and cooling starts; r, z are cylindrical coordinates of space with the origin in the center of the can; a is radius of the can; $2l$ is total height of the can; k is thermal diffusivity; and

$$\lambda_m \text{ is } \frac{m\pi}{2l}$$

Note that $\sin \lambda_m (z + l) = 1$ in the center of the can, which results in $A_{im} = j$ (as defined by Ball *(2)* for heating curves) at the center of the container where $J_0(\mu_i r) = 1$.

The values of coefficients and constants for the first term of the expansion of Equation 1 (for a 300 x 308 can) are given in Table 1 in which $A_{11} J_0(\mu_i r) \sin \lambda_1 (z + l) = a_{11}$ was defined as the intercept coefficient for a given location.

It has been found in the past by numerous research workers that the use of the first term of the converging series in Equation 1 resulted in good approximation of experimental data with the exception of the first few

TABLE 1

Distribution of theoretical intercept coefficients (a_{ri}) of heat conduction equation, and values of can constants for 300 x 308 can

$+z^1$ (cm)	r (cm)[2]			
	0	1	2	3
0.00	2.041	1.986	1.825	1.572
1.00	1.900	1.849	1.699	1.404
2.00	1.495	1.455	1.337	1.152
2.75	1.054	1.026	0.9426	.....
$\mu_1 = 0.3294$	$\lambda_1 = 0.3740$	$(\mu^2_1 + \lambda^2_1) = 0.2484$		

[1] Distance from the central horizontal cross-section.
[2] Distance from the central vertical cross-section.

minutes of the heating and cooling phase. The deviation from experimental results during the induction period of the heating phase did not influence the sterilizing value of the process, and may be considered negligible. The reverse is true when deviations (due to the use of only the first term of the expansion) from the actual cooling curve are considered which occur in the early stages of cooling. This period will be called henceforth the "intermediate" cooling period. The later stages of the cooling were found to be well approximated by the use of the first term of the theoretical expansion. These findings were restricted to determinations at the geometric center of the container.

The investigation of the whole space of the container indicated *(12)* the same trend for the heating phase. The experimental results of this investigation extended these findings tentatively to the whole thermal process when 1512 heating curves and 1512 cooling curves were plotted on semi-log graph paper for 25 locations in the can and for six processing temperatures. With the exception of the "intermediate" cooling phase the plot resulted in straight line relationships of temperature and time for all of the locations. Such relationships are expected when only one term of the theoretical equation is retained. In view of the above, regression equations were derived for the heating and cooling (with the exception of "intermediate" cooling) curves on the assumption of linearity of the logarithm of temperature differences with respect to the time.

The objective of the present work was to determine empirically the functional relationships existing among temperature, time, and space during processing of beef at high retort temperatures.

EXPERIMENTAL PROCEDURE

Apparatus and equipment. The containers used in this experiment were 300 x 308 steel, tin-plated cans sealed under 25 in. vacuum and were processed in a custom made retort of vertical type (11½ in. inside diameter and 17½ in. height).

Temperatures were determined by means of copper-constantan thermocouples and recorded by Brown Electronic Potentiometer described and calibrated by the authors in the past *(7)*.

Design of the experiment. *Raw product.* One of the ultimate practical objectives in an investigation of canning processes is to increase the palatability of a product. The choice of raw product is of importance in achieving this objective. The raw product chosen in this investigation was round of beef (matched sets of inside, outside, and knuckle of round) canner and cutter grade. This cut is likely to yield a palatable product and at the same time it is economically acceptable to the canner. Thirty-six

matched sets of round were used during the course of the experiment, supplied weekly via Railway Express from the Armour Company plant in Chicago. The meat was shipped in special insulated containers refrigerated from within by means of cans with ice and was stored at 32° F. for periods of time not exceeding 4 days.

From each cut (inside, outside, and knuckle) 9 cylindrical pieces of approximately the size of the can used (each weighing 300 g.) were cut and used as follows:

(a) One piece of meat was sealed raw and quick-frozen at −50° F. for bacteriological determinations *(1)*;

(b) two pieces were used to measure simultaneously temperatures at 3 locations in the can and to determine shear force and amount of drained juice *(8)*;

(c) two pieces were used to measure temperatures at 4 additional locations in the can;

(d) two pieces were used for bacteriological determinations *(1)* (quick-frozen after processing); and

(e) two pieces were used for storage at 100° F.

Half of the above sample (with the exception of the raw frozen can) was processed at one of the designated temperatures to give thermal histories at 7 different locations in the can, whereas the other half was processed at another temperature. Thus every week 3 cuts were used and 6 processing runs were made at all 6 designated processing temperatures.

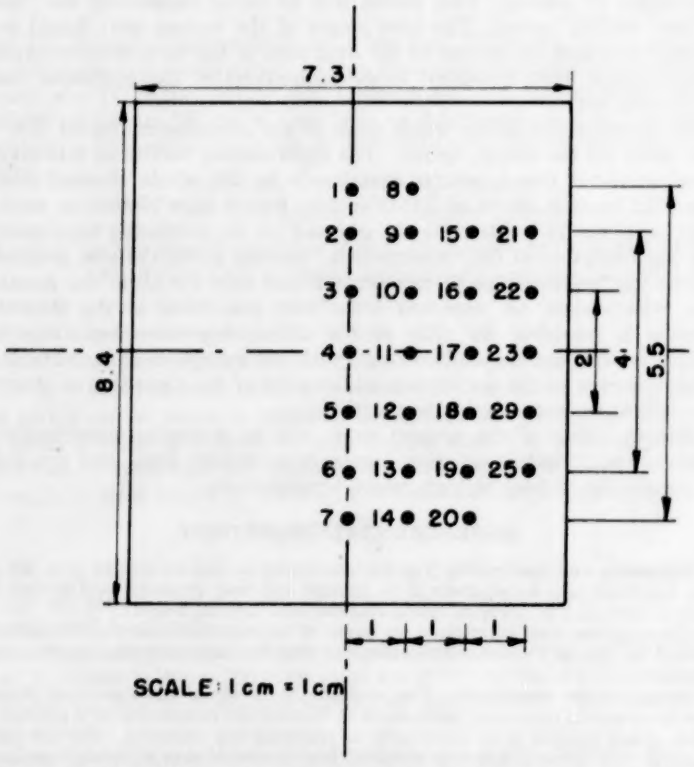

Figure 1. Distribution of locations tested during the investigation. (Vertical cross-section through the central axis of 300 x 308 cans.)

The variability of biological materials has been emphasized by many writers and in the specific case of beef a detailed discussion of real and ideal conditions was made by Hurwicz *(3)*.

To avoid variations caused by unhomogeneity of meat and by other factors affecting the raw product (such as differences in feed and age, type of cut and grade, or storage conditions) the investigation was limited to only 3 cuts of round of beef. One solid chunk of meat was processed per can (to reduce influence of convection) with its fibers parallel to the central vertical axis of the container. The storage period was held uniform throughout the experiment. Furthermore, all temperature treatments were used on one round from one animal.

Statistical design. Effects of processing temperatures, processing times, and combinations thereof on thermal, thermobacteriological and other physical characteristics of processed beef were to be determined in the course of this investigation. The wide range of processing temperatures (RT) from 225-315° F. chosen, enclosed for practical purposes, the possible range to be used in canning and offered a comparison between low- and high-temperature processing. The processing times ranged from 2-24 minutes at 315° F. to 20-120 minutes at 225° F. The time-temperature combinations used were shown in Figure 1 of Part III of this series *(8)*. The times corresponding to the time set values — T were calculated on the basis of previous research *(4)* on beef (shoulder clod) sealed in No. 2 cans which furnished some data on F_o value, and so proportionally lower processing times were assigned to higher processing temperatures to yield the same F_o value within one time set. Since the data available covered only a small portion of the range considered for this experiment, extrapolation to higher temperatures was necessary. Figure 1 *(16)* was established partially from the above considerations. Since the raw material and containers used by Bard and Tischer *(4)* differed from those used in the present investigation, and since extrapolations had to be made to obtain estimates of F_o values at higher temperatures, it was decided to use Bard and Tischer's data only as a rough guide for time set value determination. Two-minute intervals at 315° F. retort temperature from 2 to 24 minutes, and ten-minute intervals at 225° F. retort temperature from 10 to 120 minutes, were taken in actuality at the extremes of the temperature range to serve as end points for the time set values from 1 to 12. The times corresponding to other temperatures were interpolated from the lines connecting the end points for each of the time set values. The details of the design were shown in Tables 1 and 2 of Part III of this series *(16)*.

The information on the effects of individual cuts had to be sacrificed to obtain relatively more information on the effects of temperature and time on the properties of the whole round, because of the limited number of samples which could be taken from one round of beef (one animal) or from one of the cuts composing a round, and because of a wide range of temperatures and times to be used. Therefore, for every time set value the effects of all temperatures were tested for the 3 cuts together, but not all temperature-time effects were tested within every individual cut. This disadvantage of the design was compensated by the fact that it could be used on the row-by-row basis and could be interrupted at any row without complicating the interpretation of the results and detracting from the validity of the conclusions. The possibility of interruption at any time had to be considered since no information was available as to how far one should go in increasing the processing times to obtain useful information.

The statistical model of the design which may be interpreted as an incomplete block design is as follows:

$$y_{ij(k)} = \mu_{(k)} + s_{ij}[b_{i(k)} + T_{j(k)}] + e_{ij(k)}$$

where $s_{ij} = 1$ if T_j occurs in block i

and $s_{ij} = 0$ if T_j does not occur in block i, or

$$\begin{bmatrix}\text{attribute value}\\ \text{for the row}\end{bmatrix} = \begin{bmatrix}\text{mean of}\\ \text{the row}\end{bmatrix} + s_{ij}\left[\begin{matrix}\text{block effect}\\ \text{in the row}\end{matrix} + \begin{matrix}\text{treatment effect (time-temp.}\\ \text{combination in the row)}\end{matrix}\right] + \begin{matrix}\text{exp. error}\\ \text{in the row}\end{matrix}$$

and the analysis can be made row by row (representing the time set value). In this case the retort time-temperature interactions do not have to be evaluated separately as each time-temperature combination (a treatment T) is considered individually. The advantage of such an analysis lies in the fact that any attribute such as shear force, drained juice or sterilization value may be evaluated in terms of its deviation from the

postulated equivalency in terms of a time-set value or attribute $A = f(T) = f(t, T)$. Once a functional relationship is established for the attribute A (e.g., shear force) in terms of the variables involved (of whatever mathematical form it might be) and the time set values determined to result in equivalent values of this attribute for different combinations of variables, the observed deviation from equivalency can be tested by standard statistical methods in a row-by-row analysis.

Heat processing. *Technique.* The experimental routine techniques followed during the investigation of thermal characteristics of beef during heat processing were basically the same as followed by Hurwicz in previous research *(4)*, with some modification.

The cut of meat was trimmed of excessive fat, skin, and connective tissue; the 300 x 308 cans were filled as described above. The assembly of can and thermocouples was made. Net weight of meat was recorded, and then the weight of meat and assembled can was also determined before and after sealing. The thermocouples in the can were connected to the potentiometer wires, and the retort closed. The time pattern temperature and pressure controls including the automatic timer were put into operation, and all vents were closed (the drain was left partially open to allow for removal of the condensate). Venting during the processing was through the vent valve activated automatically by the controls. The length of the come-up and blow-down time (cooling under pressure) was regulated by the time patterns designed for the process, and the processing time by the automatic timer setting. The controls were shut off, and processing terminated when the temperature of the point of greatest heating lag was below 100° F. The retort was opened and drained, and the cans weighed to check for possible losses or gains during the processing period. The curves from potentiometer charts were transcribed and then replotted in rectangular and semilogarithmic coordinates. Thermal diffusivities were determined from the heating curves.

TABLE 2

Intercept coefficients ($\hat{a}_{11}$) for six retort temperatures determined by regression method

Location	Retort Temperature (°F.)						
	225	243	261	279	297	315	Average
1	0.732	0.907	0.829	1.560	1.575	4.091	1.619
7	0.805	0.759	1.209	0.974	1.005	1.256	1.001
8	1.008	1.235	1.320	1.564	1.101	1.552	1.297
14	0.373	1.409	1.572	1.095	1.300	1.232	1.164
20	0.735	1.145	1.670	1.523	1.106	1.594	1.296
21	0.767	0.821	0.814	0.816	1.110	1.352	0.947
22	0.454	0.997	1.056	1.130	1.229	0.917	0.964
23	0.810	1.032	1.087	0.964	1.167	0.855	0.986
24	0.611	0.903	1.165	1.059	1.322	0.853	0.986
25	0.697	0.643	0.554	0.937	1.351	0.663	0.808
2	1.069	1.334	1.622	1.348	2.241	1.541	1.526
6	0.902	1.533	1.673	1.473	1.310	1.324	1.369
9	1.075	1.401	1.886	1.613	1.708	2.141	1.637
13	1.618	1.890	1.923	1.355	2.390	1.600	1.798
15	0.775	1.442	1.605	1.294	1.112	1.209	1.240
16	1.348	2.082	1.653	1.601	1.241	1.211	1.524
17	1.267	1.363	2.230	2.109	1.521	1.261	1.625
18	1.438	1.592	1.859	1.434	1.336	1.260	1.487
19	1.288	2.123	1.456	1.449	1.231	1.304	1.475
3	1.380	1.355	1.729	1.620	1.465	1.083	1.439
4	1.463	1.3448	1.6295	1.4535	1.4453	1.2860	1.437
5	1.959	2.272	2.339	2.022	1.414	1.793	1.967
10	1.240	1.307	1.255	2.813	1.930	1.669	1.707
11	1.109	1.353	1.724	1.374	1.321	1.248	1.355
12	1.496	1.615	2.514	1.413	2.081	1.527	1.774

TABLE 3

Distribution of intercept coefficients ($\hat{a}_{ii}$) in 300 x 308 can

r (cm)	z (cm)						
	2.75	2.00	1.00	0.00	−1.00	−2.00	−2.75
				RT = 225° F.			
0	0.732	1.069	1.380	1.464	1.959	0.902	0.805
1	1.008	1.075	1.249	1.109	1.496	1.618	0.373
2	.....	0.775	1.348	1.267	1.438	1.288	0.735
3	.....	0.767	0.454	0.810	0.611	0.697	.....
				RT = 243° F.			
0	0.907	1.334	1.355	1.345	2.272	1.533	0.759
1	1.235	1.401	1.307	1.353	1.615	1.890	1.409
2	.....	1.442	2.082	1.363	1.592	2.123	1.145
3	.....	0.821	0.997	1.032	0.903	0.643	.....
				RT = 261° F.			
0	0.829	1.622	1.729	1.629	2.339	1.673	1.209
1	1.320	1.886	1.255	1.724	2.514	1.923	1.572
2	.....	1.605	1.658	2.230	1.859	1.456	1.670
3	.....	0.814	1.056	1.087	1.165	0.554	.....
				RT = 279° F.			
0	1.580	1.348	1.620	1.453	2.022	1.473	0.974
1	1.564	1.613	2.813	1.374	1.413	1.353	1.095
2	.....	1.294	1.601	2.109	1.434	1.449	1.523
3	.....	0.816	1.130	0.964	1.059	0.937	.....
				RT = 297° F.			
0	1.575	2.241	1.465	1.445	1.414	1.310	1.005
1	1.101	1.708	1.930	1.321	2.081	2.390	1.300
2	.....	1.112	1.241	1.521	1.336	1.231	1.106
3	.....	1.110	1.229	1.167	1.322	1.351	.....
				RT = 315° F.			
0	4.091	1.541	1.085	1.286	1.793	1.324	1.256
1	1.552	2.141	1.689	1.248	1.527	1.600	1.232
2	.....	1.209	1.211	1.261	1.260	1.304	1.594
3	.....	1.352	0.917	0.853	0.853	0.663	.....

Conditions. The conditions during the experimental runs were maintained as follows:

(a) times and temperatures were used according to the schedule in the experimental design;

(b) raw material was prepared and used as indicated under technique above; and the head space in the can was negligible when packing;

(c) method of pack used was cold pack under 25″ of vacuum;

(d) meat before packing was stored at 32° F. for not longer than a four-day period, and after sealing of meat in cans, the cans were kept in a 50-60° F. water bath to assume uniform temperature distribution within the can;

(e) cooling water temperature was between 50-70° F;

(f) temperatures at 25 locations in the can were determined. The distribution of points is shown in Figure 1. The length of the thermocouples used was always greater than or equal to the radius of the can. Thermocouples were always inserted from the side wall of the container.

RESULTS AND DISCUSSION

Heating phase. The theoretical equation for the heating phase ($0<t<t_1$) is of the following form (from Eq. 1) in its first term approximation:

$$(RT - CT) = (RT - IT)\, A_{11}\, J_0(\mu_1 r) \sin \lambda_1 (z + l)\, e^{-k(\mu^2_1+\lambda^2_1)t} \qquad \text{Eq. 2a}$$

$$(RT - CT) = (RT - IT)\, a_{11}\, e^{-b't} \qquad \text{Eq. 2b}$$

It may be transformed into

$$\log (RT - CT) = \log [a_{11} (RT - IT)] - b_h\, t \qquad \text{Eq. 2c}$$

where $b_h = b' \log e$ is the slope of the heating curve on semi-log graph and a_{11} may be called the intercept coefficient of the curve and varies from location to location.

Regression equations were derived for all of the 25 locations tested by determination of the intercept and of the slope by the least squares method. The values of the calculated parameters were defined as $\log [\hat{a}_{11} (RT - IT)]$

TABLE 4

Lag coefficients ($C_i = \hat{a}_{11}/a_{11}$) for six retort temperatures

Location	Retort Temperature (°F.)						
	225	243	267	279	297	315	Average
1	0.694	0.881	0.787	1.499	1.494	3.881	1.536
7	0.764	0.720	1.147	0.924	0.954	1.192	0.950
8	0.982	1.204	1.287	1.524	1.744	1.513	1.376
14	0.364	1.373	1.532	1.067	1.267	1.201	1.134
20	0.779	1.214	1.771	1.615	1.173	1.690	1.374
21	0.666	0.713	0.707	0.708	0.964	1.174	0.822
22	0.310	0.681	0.721	0.772	0.839	0.626	0.658
23	0.515	0.656	0.691	0.613	0.742	0.544	0.627
24	0.417	0.617	0.796	0.773	0.903	0.583	0.682
25	0.605	0.558	0.481	0.813	1.173	0.576	0.701
Average	0.610	0.860	0.992	1.031	1.125	1.298	0.986
2	0.738	0.892	1.085	0.902	1.499	1.031	1.024
6	0.603	1.025	1.119	0.985	0.876	0.886	0.916
9	0.739	0.963	1.296	1.109	1.174	1.471	1.125
13	1.112	1.299	1.322	0.931	1.643	1.100	1.235
15	0.580	1.079	1.200	0.968	0.832	0.904	0.927
16	0.793	1.225	0.976	0.942	0.730	0.713	0.897
17	0.694	0.747	1.222	1.156	0.833	0.691	0.891
18	0.846	0.937	1.094	0.844	0.796	0.742	0.875
19	0.963	1.588	1.088	1.884	0.921	0.975	1.237
Average	0.785	1.084	1.156	1.080	1.033	0.946	1.014
3	0.726	0.713	0.910	0.853	0.771	0.571	0.757
4	0.717	0.659	0.798	0.712	0.708	0.630	0.704
5	1.031	1.196	1.231	1.064	0.744	0.944	1.035
10	0.676	0.707	0.679	1.521	1.044	0.913	0.923
11	0.558	0.681	0.868	0.692	0.665	0.628	0.682
12	0.809	0.873	1.360	0.764	1.125	0.826	0.960
Average	0.753	0.803	0.974	0.934	0.843	0.752	0.843
Grand Average	0.712	0.900	1.021	0.990	0.985	0.985	0.932

and f_h respectively. The regressions were computed for the average experimental values of log (RT − CT) for 9 curves (3 curves for each of the 3 cuts of the round of beef). The readings at t = 0 were disregarded and regression computations were made only for the linear part of the curve observed from the plot, thus eliminating the possible influence of thermal lag at the beginning of the heating on the value of the slope.

The time of processing (t_1) was not equal for all of the 9 curves which resulted in unequal weights for the averages used in the regression. The equations thus derived approximate the experimental data best for shorter processing times. Since the experiment was planned for investigation of high temperature processes which give sufficient sterilizing effect for relatively short times, this method was found satisfactory for the useful range of processing times at the temperatures used.

It was also observed that the slope values computed for very short processes were relatively smaller than those for longer runs, thus reflecting the

TABLE 5

Distribution of lag coefficients (C_1) in 300 x 308 can

r (cm)	z (cm)						
	2.75	2.00	1.00	0.00	−1.00	−2.00	−2.75
	RT = 225° F.						
0	0.694	0.738	0.726	0.717	1.031	0.603	0.764
1	0.982	0.739	0.676	0.558	0.809	1.112	0.364
2	.....	0.580	0.793	0.694	0.846	0.963	0.779
3	.....	0.666	0.310	0.515	0.417	0.605	.....
	RT = 243° F.						
0	0.861	0.892	0.713	0.658	1.196	1.025	0.720
1	1.204	0.963	0.707	0.681	0.873	1.299	1.373
2	.....	1.079	1.225	0.747	0.937	1.588	1.214
3	.....	0.713	0.681	0.656	0.617	0.558	.....
	RT = 261° F.						
0	0.787	1.085	0.910	0.798	1.231	1.119	0.147
1	1.287	1.296	0.679	0.868	1.360	1.322	1.532
2	.....	1.200	0.976	1.222	1.094	1.089	1.771
3	.....	0.707	0.721	0.691	0.796	0.481	.....
	RT = 279° F.						
0	1.499	0.902	0.853	0.712	1.064	0.985	0.924
1	1.524	1.109	1.521	0.692	0.764	0.931	1.067
2	.....	0.968	0.942	1.156	0.844	1.084	1.615
3	.....	0.708	0.772	0.613	0.723	0.813	.....
	RT = 297° F.						
0	1.494	1.499	0.771	0.707	0.774	0.876	0.954
1	1.073	1.174	1.044	0.665	1.125	1.643	1.267
2	.....	0.832	0.730	0.833	0.786	0.921	1.173
3	.....	0.964	0.839	0.742	0.903	1.173	.....
	RT = 315° F.						
0	3.881	1.031	0.571	0.630	0.944	0.886	1.192
1	1.515	1.471	0.913	0.628	0.826	1.100	1.201
2	.....	0.904	0.713	0.691	0.742	0.975	1.690
3	.....	0.906	0.702	0.296	1.213	0.423	.....

influence of the initial thermal lag period which does not properly belong to the linear portion of the heating curve. A separate regression calculation for each heating curve could have introduced bias in determination of the constants of the equations and resulted in less reliable estimates than those afforded by the procedure adopted above. The estimates of variance obtained must be considered as estimates of variation of means of nine determinations.

The values of $\hat{a}_{11}$ obtained at all the locations and temperatures have been tabulated in Tables 2 and 3. The lag coefficient (Tables 4 and 5) (or correction factor for the theoretically expected a_{11}) was evaluated as $C_1 = \hat{a}_{11}/a_{11}$ and may be used in connection with Equation 2.

The values of the slopes $\hat{b}_h$ for all the processing conditions encountered during this investigation were found to be substantially the same for all locations in the can but varying with the processing temperature. The aver-

TABLE 6

Slopes ($\hat{b}_h$) of heating curves and their variation

RT (°F.)	225	243	261	279	297	315	Average
$\hat{b}_h \times 10^2$ (min.)$^{-1}$	23.75	27.29	30.27	29.58	29.62	26.24	27.79
C.V. ($\hat{b}_h$) (%)	7.72	4.57	5.69	5.06	6.02	9.32	6.40
C.V. (RT − CT) (%)	33.16	18.44	21.00	16.79	17.63	11.66	19.78
C.V. (RT − IT)a_{11} (%)	10.96	5.72	7.80	5.51	6.10	4.78	6.81

age values of $\hat{b}_h$ are given in Table 6 for the 6 retort temperatures and may be used in connection with Equation 2 (c) with the corrected a_{11} to determine the temperatures at any location in the can for the retort temperature range 225-315°F. The coefficient of variation of $\hat{b}_h$ is given in Table 6 and did not exceed 9.32%. In the same table the standard error of estimate is given in the determination of log (RT−CT). This error was expressed as percentage of (RT−CT) (*17*, p. 248) and its average was 19.78%. Table 6 indicated better approximation of the data by the regression equations for higher retort temperatures.

The heating equation describing the thermal histories of the can contents is as follows:

$$(RT - CT) = (RT - IT)\, C_1\, a_{11}\, e^{-\hat{k}(\mu'_1 + \lambda'_1)\,t} \qquad \text{Eq. 3a}$$

or with average values of the factors determined during this investigation for 300 x 308 can

$$(RT - CT) = (RT - IT)\, 0.934\, a_{11}\, e^{-0.065184} \qquad \text{Eq. 3b}$$

The departures from the theoretical expectation will be reflected best if the values for C_1 are taken from Table 4 for each location and temperature separately, with the average value of $\hat{k}$ (Fig. 2) being taken from the curve representing $k = f(RT)$.

As a general approximation of the thermal history of the can contents it will be sufficient to use Equation 3b with the average $\hat{k}$ and C_1 given in Table 7. This table also indicates the average coefficients of variation for these characteristics.

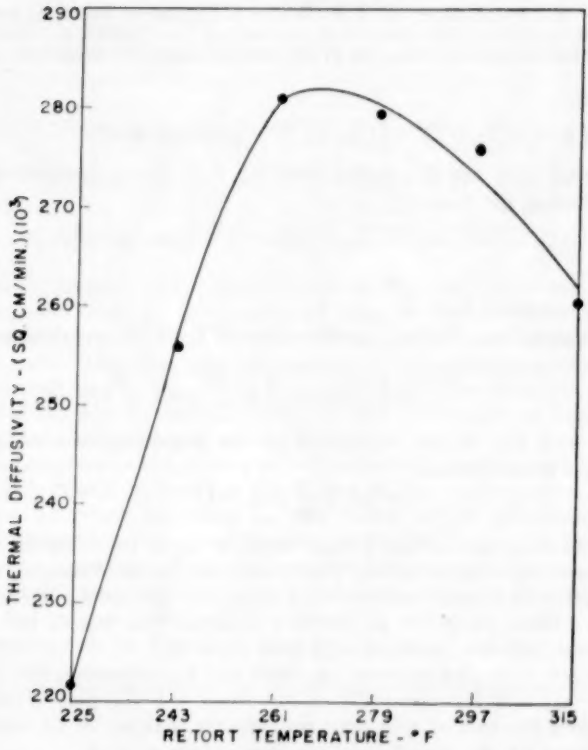

Figure 2. Relationship between thermal diffusivity and retort temperature.

Cooling phase. The exact analytical solution of the time-temperature relationship for the cooling phase (Eq. 1) in its first term approximation may be transformed as follows:

$$CT = RT - (RT - CT)_{t_i} + (TC - RT) - (TC - RT)\, a_{11}\, e^{-b'(t-t_i)} \qquad \text{Eq. 4a}$$

$$CT = -(RT - CT)_{t_i} + TC - (TC - RT)\, a_{11}\, e^{-b'(t-t_i)} \qquad \text{Eq. 4b}$$

$$(CT - TC) + (RT - CT)_{t_i} = (RT - TC)\, a_{11}\, e^{-b'(t-t_i)} \qquad \text{Eq. 4c}$$

TABLE 7

Average coefficients and parameters to be used with prediction equation for heating phase (Eq. 3a) and their variation

RT (°F.)	225	243	261	279	297	315	Average
$\hat{k} \times 10^5$ (sq. cm/min.)	221.63	255.80	281.08	279.46	275.79	259.97	262.29
C.V. ($\hat{k}$) (%)	7.72	4.57	3.69	5.06	6.02	9.32	6.40
C.V. ($\hat{a}_{11}$) (%)	10.46	5.86	6.60	5.47	4.56	5.28	6.37
$C_i = \hat{a}_{11}/a_{11}$	0.708	0.895	1.020	0.992	0.990	0.296	0.934

Since $RT - TC = [(CT_{t_1} - TC) + (RT - CT)_{t_1}]$ or $= (m + g)$ and $b' = \frac{1}{f_h \log e}$ as well as $(t - t_1) = x_{el}$, in Ball's terminology the equation may be expressed as:

$$(CT - TC + g) = (CT_{t_1} - TC + g)\, a_{11}\, e^{-b'(t-t_1)} = (m+g)\, a_{11}\, e^{(x_{el}/f_h)} \qquad \text{Eq. 4d}$$

and compared with the expression used by Ball *(2)* to approximate the cooling curves of the form:

$$(CT - TC) = j\,(CT_{t_1} - TC)\, e^{-b''(t-t_1)} = mje^{(x_{el}/f_{el})} \qquad \text{Eq. 5a}$$

in which he assumed $f_{el} = f_h$
if the exact equation (Eq. 4d) is solved for $(CT - TC)$ one obtains

$$\begin{aligned}(CT - TC) &= a_{11}\, me^{x_{el}/f_h} + a_{11}\, g\, e^{x_{el}/f_h} - g \\ &= a_{11}\, me^{x_{el}/f_h} + g\,(a_{11}\, e^{x_{el}/f_h} - 1)\end{aligned} \qquad \text{Eq. 5b}$$

If Eq. 5a and Eq. 5b are compared at the beginning of cooling when $(t - t_1) = x_{el} = 0$ one obtains

$$j = a_{11} + \frac{g}{m}\,(a_{11} - 1) \qquad \text{Eq. 6a}$$

which conclusively proves that j is not equal to a_{11} at the center of the container as used for cooling curves. This points out the inconsistency of using j determined from heating curves at can center for application to the cooling curves since there $j = A_{11} = a_{11}$ by its definition. This was to be expected since j determines the intercept on y-axis when $(t - t_1) = x_{el} = 0$ for the $(CT - TC) = f\,(t)$ plot on semi-log paper and a_{11} determines the intercept when $(t - t_1) = 0$ for the $[(CT - TC) + g] = f\,(t)$ plot. Thus j was found to be a function of g/m ratio and greater than a_{11} in all locations in the container with the exception of locations where $a_{11} = 1$.

At any time of cooling j may be determined from the equality of the equations 5a and 5b with $E_{el} = e^{(x_{el}/f_{el})}$ and $E_h = e^{x_{el}/f_h}$

as $$j = a_{11} + \frac{g}{mE_{el}}\,(a_{11}\, E_h - 1) \qquad \text{Eq. 6b}$$

The above relationship solved for E_{el} with the value of j obtained from Eq. 6a.

$$E_{el} = \frac{(a_{11}\, E_h - 1)}{(a_{11} - 1)} = e^{x_{el}/f_{el}} = \frac{(a_{11}\, e^{x_{el}/f_h} - 1)}{(a_{11} - 1)}$$

indicates that f_{el} is a function of a_{11} and f_h but cannot be equal to f_h as assumed by Ball. If the assumption is made that $f_{el} = f_h$ then $E_{el} = E_h = 1$, which can occur only when $(t - t_1) = x_{el} = 0$ and at no other time during the cooling phase. The analysis of variance made for $\hat{f}_h$ and $\hat{f}_{el}$ values determined by regression methods confirmed *(10)* these theoretical expectations and showed in addition that $\hat{f}_{el}$ and $\hat{f}_h$ values are not affected by either processing temperature or location in the can.

The cooling curves were evaluated by regression method in the form of

$$\log (CT - TC) = \log [j\,(CT_{t_1} - TC)] - b_{el}\,(t - t_1) \qquad \text{Eq. 5c}$$

where $b_{el} = b'' \log e$

TABLE 8

Average coefficients and parameters to be used with prediction equations for the cooling phase (Eqs. 3a, 3b, and 4b) and their variation

RT (°F.)	225	243	261	279	297	315	Average
$\hat{b}_{cl} \times 10$ (min.)$^{-1}$	26.95	25.22	25.97	24.52	25.28	24.91	25.48
C.V. ($\hat{b}_{cl}$) (%)	12.31	19.02	42.53	36.47	25.33	19.26	25.82
C.V. (CT − TC) (%)	6.08	9.33	17.83	13.38	10.72	8.00	10.90
C.V. ($CT_{t_1} - TC$)$\hat{j}$ (%)	7.35	3.46	23.96	18.41	14.04	9.64	12.81
$C_2 = \hat{a}'_{11}/\hat{a}_{11}$	1.329	0.839	0.848	0.373	0.865	0.893	0.941
$C_3 = \hat{j}/j$	0.873	0.730	0.802	0.873	0.867	0.755	0.817

in the same manner as the heating curves. The regression computations, however, were made on the average of three curves (for the three cuts of beef) instead of nine, since the value of $\hat{j}$ was found to be dependent on the g/m ratio. Thus the average was taken from three curves which displayed approximately the same g/m values. Again the linear portion of the curves was estimated from their semi-log plot to exclude the data from the intermediate cooling phase.

The regression calculations yielded values for the slopes of the cooling curves ($\hat{b}_{cl}$) (Table 8) and for the values of the cooling curve intercept coefficient ($\hat{j}$). Since the slope $\hat{b}_{cl}$ was found not to be affected by the temperatures or locations *(10)* its average (0.025475) may be used in connection with Equation 5(b) for all conditions encountered in this investigation. The computed values of $\hat{j}$ were compared with the theoretical j and with $\hat{j}_1$ derived by means of Equation 6(a) using $\hat{a}_{11}$ determined from heating equations. By means of the same equation $\hat{a}'_{11}$ was computed from

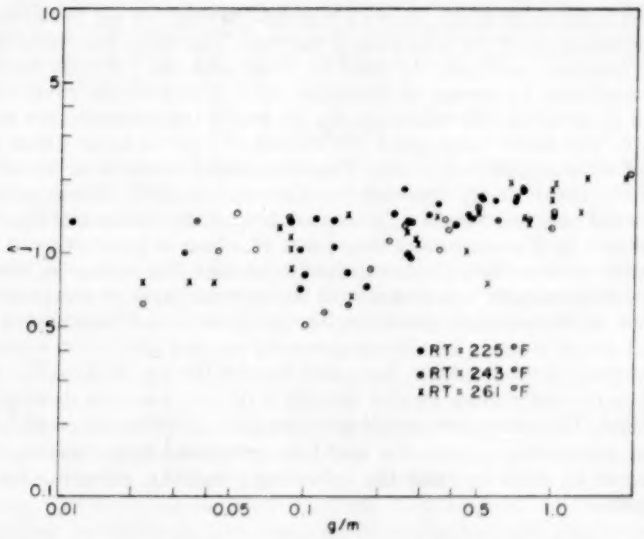

Figure 3. Scatter diagram in log-log coordinates for the j and g/m relationship at low retort temperatures.

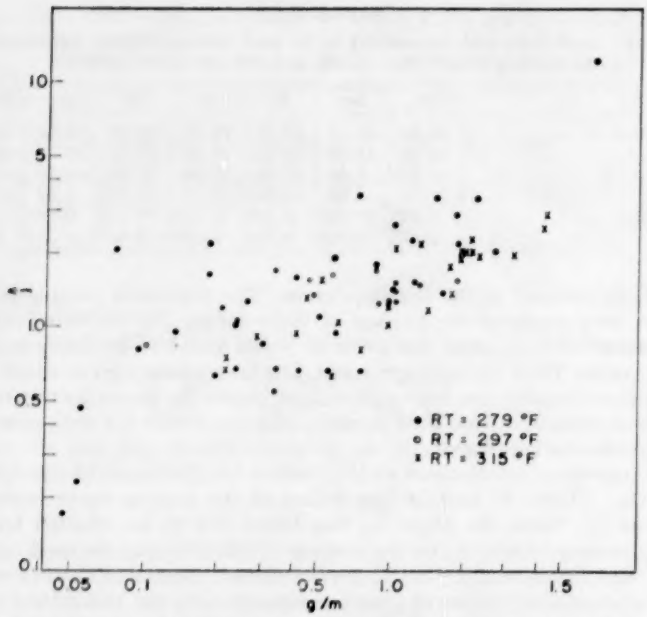

Figure 4. Scatter diagram in log-log coordinates for the j and g/m relationship at high retort temperature.

$\hat{j}$ values and the ratio of $\hat{a}'_{11}/\hat{a}_{11} = C_2$ was determined for all the processing conditions at all of the locations in the can. This ratio was found substantially constant and may be used to determine the $\hat{j}$ if only heating data are available by means of Equation 6(a). The average value of C_2 was found to be 0.941. Its values for the six retort temperatures are given in Table 8. The same table gives the values of $\hat{j}/j = C_3$ ratio which also was found substantially constant. This ratio may serve as a correction factor for the theoretically expected j and is equal to 0.817. The computed $\hat{j}$ were plotted as a function of g/m and confirmed the theoretical expectation expressed in Equation 6(a) that $\hat{j}$ is a function of g/m. (Figs. 3 and 4.) The plot resulted in an approximately straight line in log-log coordinates. The slope seemed to increase with the increase in retort temperature.

In view of the apparent constancy of the C_2 ratio and dependency of $\hat{j}$ on g/m, it would seem to be advantageous to use as a prediction equation for the cooling phase Equation 4(c) and to plot the log $(CT - TC + g)$ against $(t - t_1)$ rather than to plot the log $(CT - TC)$ as it is done at the present time. This procedure would save separate determinations of $\hat{f}_h$ and $\hat{f}_{cl}$ and of computing $\hat{j}$; also $\hat{a}'_{11}$ could be estimated from heating data by means of C_2 ratio to yield the following prediction equation for the cooling period.

$$(CT - TC) + (RT - CT)_{t_1} = (RT - TC)\, C_2\, \hat{a}_{11}\, e^{-\hat{k}(\rho^2_1 + \lambda^2_1)(t - t_1)} \quad \text{for } (t - t_1) > 10 \qquad \text{Eq. 7a}$$

with $\hat{a}_{11}$ estimated from heating data. This equation would take into account all departures from theory during the heating period. Equation 7(a) combined with the prediction equation for the heating period (Eq. 3a) would give a general relationship of the following form:

$$(CT-TC)+(RT-CT)_{t_1}=(RT-TC)\,C_2\,C_1\,a_{11}\,e^{-\hat{k}(\mu^2_1+\lambda^2_1)(t-t_1)} \qquad \text{Eq. 7b}$$

The factors in the above equation can be computed either on the basis of theoretical considerations (μ^2_1; λ^2_1; a_{11}) or from the results of this investigation (C_1; C_2 and $\hat{k}$). The average factor $C_2=0.941$ for all temperatures, and the factor C_1 to be used may be taken from Table 7; if only an approximate relationship is found sufficient, the average $C_1=0.934$ may be used. The values of $\hat{k}$-thermal diffusivity as in the case of heating equation may be taken from Fig. 2 for corresponding retort temperatures or an average value of $\hat{k}=0.2623$ sq cm/min may be used. Thus the average time-temperature relationship during cooling phase for 300 x 308 cans with beef is as follows:

$$(CT-TC)+(RT-CT)_{t_1}=(RT-TC)\,0.879a_{11}\,e^{-0.06515(t-t_1)} \quad \text{for } (t-t_1)>10 \qquad \text{Eq. 8a}$$

The alternate equation of the form used by Ball would be

$$(CT-TC)=(CT_{t_1}-TC)\,C_3\,je^{-0.05563(t-t_1)} \text{ for } (t-t_1)>10 \qquad \text{Eq. 8b}$$

and where j is computed from Eq. 6(a) and average $C_3=0.817$.

The average variation of the log [(CT−TC)] as determined from regression of means of three curves is shown in Table 8, and the average C.V. was 10.90% of (CT−TC). The average coefficients of variation of $\hat{b}_{el}$ are presented in Table 8; and the average C.V. was 25.82%, considerably larger than the variation in $\hat{b}_h$. The average C.V. of $\hat{j}$ $(CT_{t_1}-TC)$ was found to be 12.81%. The larger variation in $\hat{b}_{el}$ was reflected also in the analysis of variance of $\hat{f}_{el}$ *(5)*.

SUMMARY

An investigation of time, temperature, and space relationship during canning of beef extended to high retort temperatures (up to 315°F) was made. Thermal histories were obtained for 25 locations in 300 x 308 cans packed with round of beef. The functional relationships were determined by regression methods for the heating and cooling phases of processing with the exception of first ten minutes of the cooling ("intermediate" cooling). Physical properties of beef not available in the literature have been ascertained.

In addition to the establishment of empirical relationships by means of the determination of correction factors for the theoretical relationship for the whole space of the can (not only the geometrical center) for both heating and cooling phases, a method has been devised to obtain empirical constants for the cooling phase from experimental data gathered during heating. The experimental work thus has been reduced for future research with other commodities. The precision of results has been evaluated by the estimate of experimental errors associated with them.

On the basis of the experimental results obtained during the investiga-

tion and for the processing conditions prevailing during the experiment the following conclusions can be drawn:

The average time-temperature relationship existing in the can is expressed best during the heating phase of thermal process ($t<t_1$) by the expression below:

$$(RT - CT) = (RT - IT)\, 0.934\, a_{11}\, e^{-0.06515t}$$

and during the cooling phase ($t - t_1 > 10$) by the following expression:

$$(CT - TC) + (RT - CT)_{t_1} = (RT - TC)\, 0.879\, a_{11}\, e^{-0.06515(t-t_1)}$$

The availability and manner of determination of the above empirical time-temperature-space relationship should lead to improvement of process calculations and to the increase in their precision and reliability. Ultimately it should become a part of the overall correlation of fundamental laws governing the process variables and thus contribute to the development of not only safe but also palatable products.

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HEAT PROCESSING OF BEEF. V. TEMPERATURE DISTRIBUTION PATTERNS DURING PROCESSING OF BEEF AT HIGH RETORT TEMPERATURES[a]

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The modern trend in evaluation of lethality of canning processes *(1, 2, 8)* is founded on the basis of thermal history of the whole can volume rather than on that of the geometrical center of the container only, and necessitates a study of temperature distribution in the container during the canning process. With the exception of the paper by Hurwicz and Tischer *(5)*, no such data pertaining to the foodstuffs are available in the literature.

The experimental temperature distributions and their analyses presented here are a part of a larger study to determine thermal, thermobacteriological, and other physical characteristics of round of beef processed at higher than conventional retort temperatures and for short processing times. The containers used were 300 x 308 cans, retort temperature range was 225-315°F. The experimental procedure was similar to that used by Hurwicz and Tischer in Part II of this series *(5)* and has been fully described in *(3)* and *(6)*.

The objective of this work was to determine the temperature distributions in the can during processing and to analyze the experimental deviations from the theoretical expectations.

EXPERIMENTAL TEMPERATURE DISTRIBUTION

The temperature distributions observed and mapped during this experiment did not meet the theoretical expectations. The expected isothermal distribution for a homogeneous isotropic body heating by conduction would be symmetrical about the vertical and horizontal axes; it would change from a cylindrical distribution on the outside of the can through an ellipsoidal form to end in a point at the center of the container or, all these forms would be taken by one isothermal surface moving from the outside toward the center of the container as the heating progressed in time. Approximately such a distribution was observed by Hurwicz and Tischer *(5)* in No. 2 cans of beef, even though some of the constants of the heating equations had to be multiplied by a correction factor applied to all locations in the container.

Mapping procedure. The locations investigated have been shown in Figure 1, and have been assumed to be located on any half-plane of the central vertical cross-section. The diagrams of the isotherms were prepared in two steps. First, 72 interpolation diagrams were prepared for each cut of round (e.g. Fig. 2). For this purpose temperatures obtained from 3 heating curves were plotted against the points located along one of the 4 vertical axes in the can. Five-minute intervals were used from the beginning to the end of the heating phase for each retort temperature (RT). Next, the temperatures were determined from the interpolation diagram (for heating periods increasing in length by 5-minute intervals) at the intersection of the horizontal tem-

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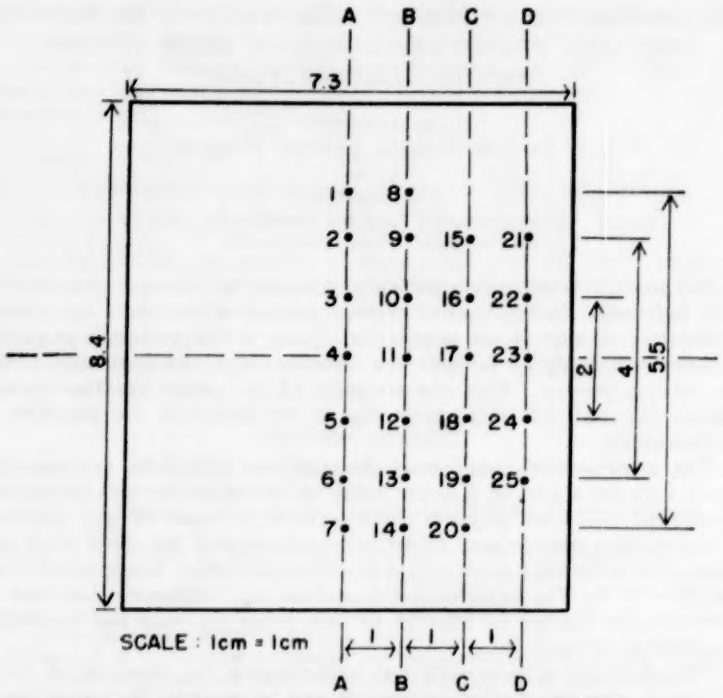

Figure 1. Distribution of locations tested during the investigation. (Vertical cross-section through the central axis of 300 x 308 cans)

perature line with the appropriate equal-time (isochronal) line. The intersection of these 2 lines defined in the space the location in the container. This procedure was repeated 4 times (once for each vertical axis: A-A, B-B, C-C, and D-D) and usually yielded 8 locations in the container at the same temperature for the heating period. The points thus located in the can were connected by an isothermal line on a diagram representing the central vertical cross-section of the can. Figures 3, 4, and 5 represent typical isothermal temperature distributions observed in the course of this experiment from 1512 heating curves at 25 locations in a 300 x 308 can of beef for 6 retort temperatures.

Occurrence of distorted temperature distribution. The isothermal temperature distributions exhibited considerable deviations from theoretical expectations. Considering the distribution of a number of isothermal lines after some time elapsed from the beginning of heating, one notices that the point of greatest heating lag* did not coincide with the geometric center of the can. The point of greatest heating lag in almost all of the derived distributions was found to be displaced from the center and located in the central horizontal cross-section of the can. Sometimes it was located below or above this plane.

The form of isothermals located in the outside region of the can did not depart from the theoretically expected, but the isothermals found in the central region instead

* This point has been commonly called "the point of slowest heating" which erroneously implies lower thermal diffusivity at this location; "the point of greatest heating lag" implies correctly the highest temperature difference at time zero on the assymptote of the heating curve.

of being ellipsoidal displayed cardioid characteristics. In the vicinity of the center, the theoretically concentric elipsoidal or spherical shells were replaced by doughnut-shaped volumes (considering the revolution of the vertical cross-section by 360° about the central axis), similar to those shown in Figures 6 and 7. The distributions appeared to be symmetric about the central horizontal plane. The same effects were observed when the changes in the space of one isothermal surface were traced at different intervals of time. These phenomena appeared to be more pronounced for high retort temperatures in the range from 279-315°F.

The area of greatest heating lag was removed further from the central axis and the isothermals assumed cardioid shape closer to the boundaries of the container.

The effect of longer heating periods on the temperature distributions was similar to the effect of higher retort temperature.

In view of the fact that these phenomena were observed at all temperatures and times, and for all of the 3 cuts of round (separately as well as for the average of all three), it seems improbable that this occurrence was only a chance event or due to the interpolation procedure. The proof of the reality of this occurrence will be given in the evaluation of the parameters of the heating equations in the following section. Meanwhile, an explanation of the mechanism of the occurrence of the distorted temperature distributions and departure from theoretical expectations will be attempted.

Mechanism of occurrence of distorted temperature distribution. Temperature distributions expected from theoretical consideration obey the law expressed in the heat conduction equation *(4)* given in Table 1. This equation holds for a homogeneous

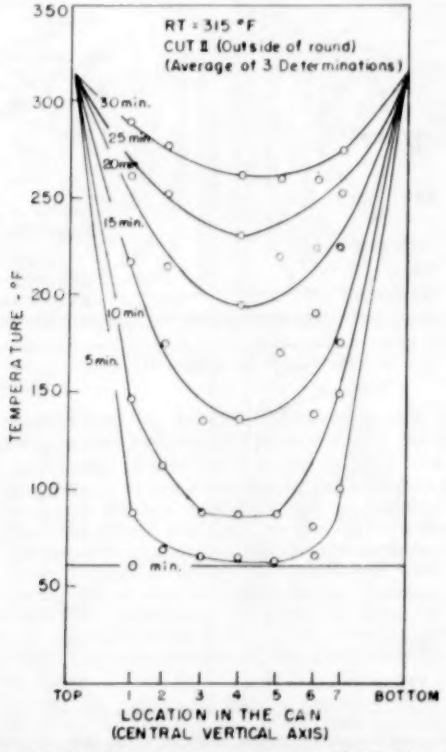

Figure 2. Interpolation diagram for temperature distribution determination. (Isochronal temperature distribution)

TABLE 1

Distribution of theoretical intercept coefficients (a_{11})[1] of heat conduction equation, and values of can constants for 300 x 308 can

$\pm x^2$ (cm.)	r (cm.)[2]			
	0	1	2	3
0.00	2.041	1.986	1.825	1.572
1.00	1.900	1.849	1.699	1.404
2.00	1.495	1.455	1.337	1.152
2.75	1.054	1.026	0.9426	.....

$\mu_1 = 0.3294$ $\lambda_1 = 0.3740$ $(\mu_1^2 + \lambda_1^2) = 0.2484$

[1] $RT - CT = (RT - IT)\, A_{11}\, J_o(\mu_1 r) \sin \lambda_1 (z + 1)\, e^{-k(\mu_1^2 + \lambda_1^2)t} = a_{11}\, e^{-k(\mu_1^2 + \lambda_1^2)t}$.

[2] Distance from the central horizontal cross-section.

[3] Distancee from the central vertical cross-section.

isotropic body, and the solution includes constants depending on the initial uniform temperature distribution. A departure from any of these assumptions will result in departure from the theoretical expectation. The material tested in this investigation

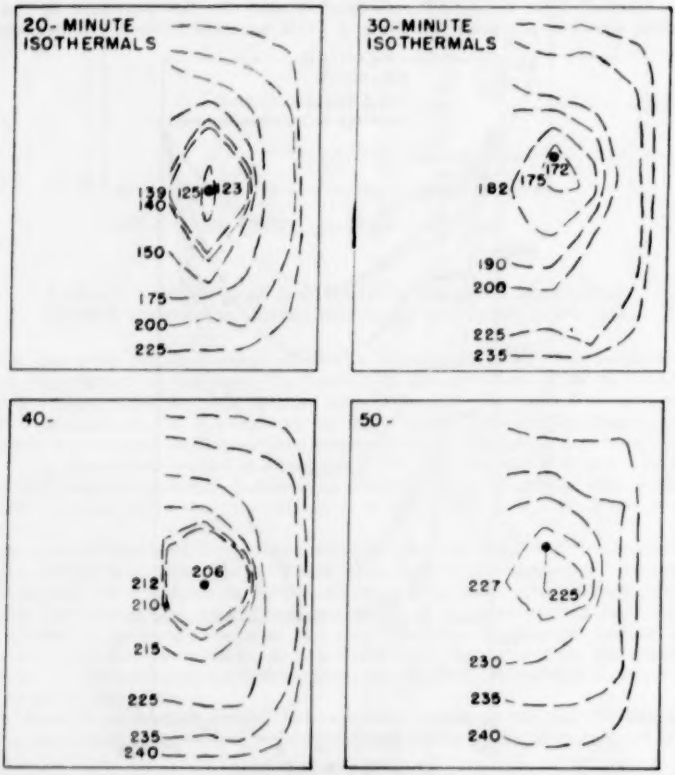

Figure 3. Isothermal temperature distributions after four intervals of time during heating at 243°F.

(Cut III—inside of the round)

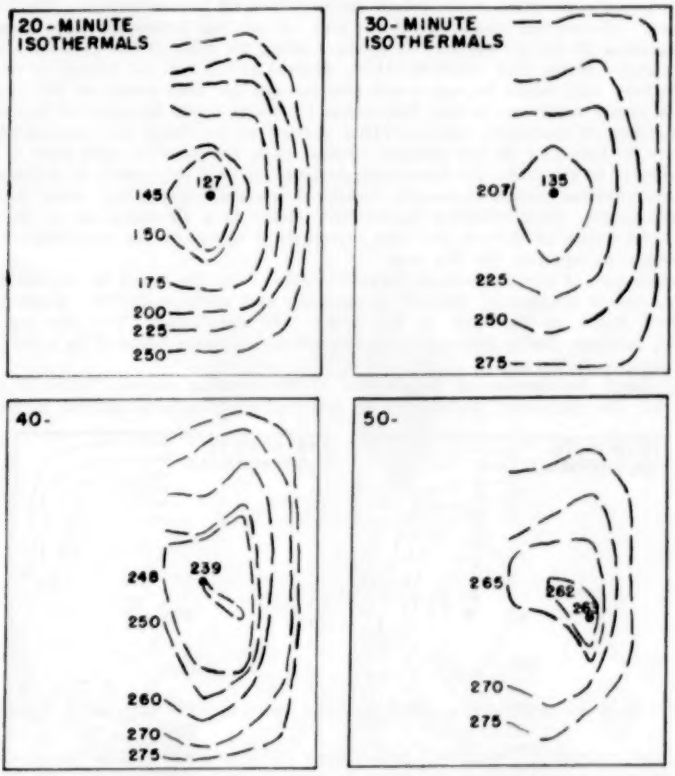

Figure 4. Isothermal temperature distributions after four intervals of time during heating at 279°F.

(Cut III—inside of the round)

(beef) is neither homogeneous nor isotropic; furthermore, its structure undergoes changes in chemical composition and moisture content during the process.

The anisotropy of the piece of beef sealed in the container with the fibers parallel to the vertical axis is evident. If the resistance to the heat flow is lower along the fibers than across them, then the heat wave front may be expected to progress faster towards the center from the bottom and from the top of the cylinder than from the sides. As a result, the two isothermal fronts, one concave upward and the other downward, meet at the center before the "side" isothermals can reach this point, and a volume of greatest heating lag of doughnut shape (torus) is formed. Figures 6 and 7 picture such occurrence. This phenomenon, which may be called an "end effect" will be more pronounced in cylinders which have a height-diameter ratio (*l*/a) of unity or less. This may explain the non-occurrence of this phenomenon in a previous experiment with No. 2 cans *(5)*.

The cardioid shape of the isothermals may be explained in the same manner by the relative velocities of the moving heat fronts and their concavities. The effect of the inherent anisotropy of beef is further accentuated by the searing effect on the sides of the cylinder of beef processed at higher temperatures; this effect constitutes a type of "casehardening" and may contribute to higher resistivity of the sides of

the cylinder of meat which would retard the movement of the isothermals. The "case-hardening" effect which was apparent for even the shortest processing times from the very beginning of the process may be stronger along the sides of the cylinder because a whole length of the fiber would be sealed, whereas at the top and bottom of the can only the fiber ends would be sealed and free passage for heat would be left between them. A higher resistance to heat flow across the fibers would be expected because of the resistivity of connective tissue running parallel to the fibers and because of less resistance to heat flow in the straight vertical pass, compared to sine path of flow perpendicular to the fiber. All these characteristics of beef may result in a formation of several effective centers of heating (points of greatest heating lag) other than the geometric center. These effective centers are located in a circumference in the horizontal cross-section of the can and may necessitate a change in the parameters of the heat conduction equation for this case.

The change, if any, in thermal diffusivity value from the center to the outside of the can, due to changes in chemical composition and moisture content, might be an additional factor in distortion of the temperature distribution. This has not been observed, however, during this experiment, as diffusivities were found to be independent of the location in the can.

Calculated distribution of parameters of the heating curves. *Intercept Coefficient—$\hat{a}_{11}$:* The theoretical distribution of intercept coefficients $\hat{a}_{11}$ defined in Table 1

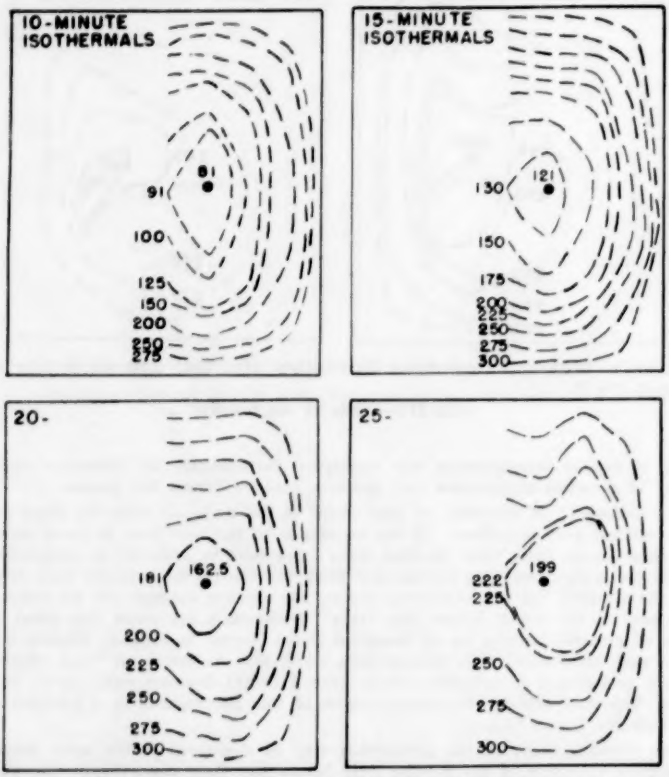

Figure 5. Isothermal temperature distributions after four intervals of time during heating at 315°F.

(Cut III—inside of the round)

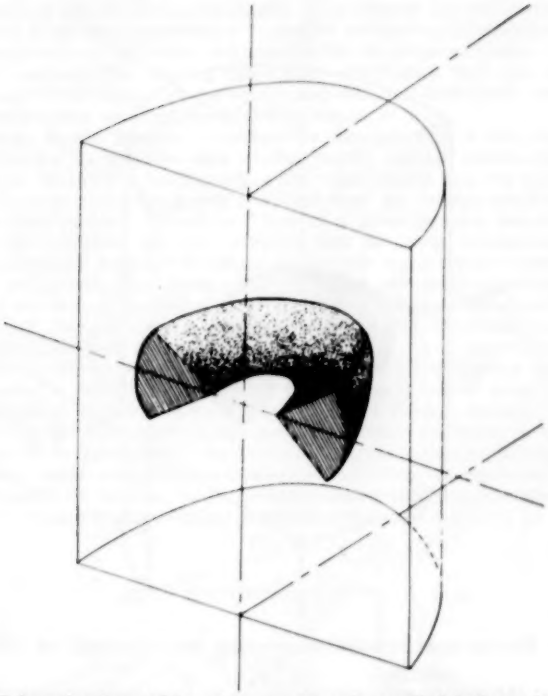

Figure 6. Triangular torus of lowest temperatures in a cylindrical can (300 x 308).

(on the assumption of uniform initial temperature distribution) indicates the greatest lag and subsequently lowest temperatures at any time of processing at the center of the container. These coefficients have the higher value at the center of the container and decrease toward the outside. The coefficients in a relationship corrected for average experimental conditions by a factor of f_s would display the same trend *(5)*. If the estimates available for the coefficients actually determined during an experiment on the basis of temperature and time measurement have a different distribution, the location where they have the highest value (experimental error taken into account) will be the location of greatest lag in heating and as such will display lowest temperatures at any time of processing. This will be true if diffusivities are the same for the whole can. Whatever the cause of the initial lag, the effects on distribution of the intercept coefficients will be as above and thus confirm the reality of distribution of temperatures mapped from experimental heating curves.

Since every measurement is subject to an experimental error the actual position of two isothermal lines relative to each other is not certain. It may be said that e.g. on Figure 4 the 20-minute isothermals are in effect 20 minutes $\pm$ 1 sec. isothermals; also, that the 145°F. and 150°F. isothermals are 145 $\pm$ 5° and 150 $\pm$ 5° isothermals. The problem is then to define in the space of the container and in the time the volume shell corresponding to the fiducial limits of the error; this in effect leads to a 5-dimensional space (X, Y, Z space coordinates and t-time and T-temperature) analysis, very difficult if at all possible to make on the basis of temperature readings. Instead, comparisons of intercept coefficients (functions of temperature, time and space coordinates) are made to ascertain statistically whether or not differences between them are real (significant). This together with actual values of the coefficients fixes their distribution as well as the distribution of temperatures. In this manner one is able to say whether

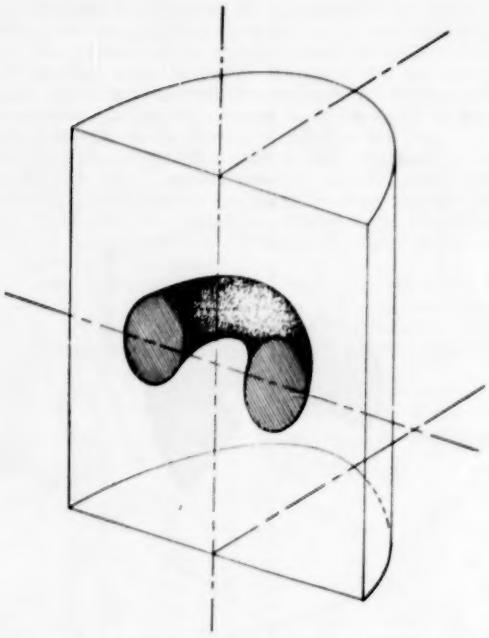

Figure 7. Circular torus of lowest temperatures in a cylindrical can (300 x 308).

the distortion of temperature is real or due to the experimental error. The value of this type of analysis will be demonstrated below where apparent shift of the point of greatest heating lag in the vertical direction indicated by experimental temperature distribution has been disproved by statistical analysis of intercept coefficients, and must be ascribed to experimental variation.

The values of intercept coefficients are based on regression calculations and as such have smaller experimental error than individual temperature readings. They reduce the infinite number of temperature distribution diagrams, which would be necessary to cover one process, to one coefficient distribution indicative of the whole process. Since they are determined from regression, the estimates of the experimental error are readily available for calculation of fiducial units and statistical comparisons. The conclusions based on coefficient distribution must be considered valid for temperature distributions since the coefficients are functions of the temperature and time, and as such offer proof of reality or nonreality of observed phenomena.

Lag Coefficient—C_1: The relative departure from the theoretically expected constants may be measured in terms of the ratio $C_1 = \hat{a}_{11}/a_{11}$ referred to henceforth as the lag coefficient, where $\hat{a}_{11}$ is determined from regression equations and a_{11} is the theoretically expected value. If the lag coefficient is smaller than unity it indicates a smaller actual lag than in the ideal case. The magnitude of this coefficient for various locations in the can will indicate the comparative (relative) departure (or deviation) from the theoretical relationship at these locations. The relatively lower values of the lag coefficient at some locations compared to others will then indicate a decrease in heating lag of larger order, compared to the theoretical, than for the other locations. Values smaller than unity compared to values larger than unity immediately indicate that temperatures respectively higher and lower than the theoretical temperatures have to be expected at any time of processing at these locations.

The use of the lag coefficient in interpretation of the data permits rapid comparison of experimental results with theoretical expectations or of conversion of theoretical temperature distributions to experimental ones if the data on $\hat{a}_{11}$ and a_{11} are available.

The interpretation may be further facilitated if an average lag coefficient is calculated which amounts to an average correction factor for the heating relationship with respect to the whole space in the container and accounts for the effect of fitting of one term of the heating equation. The average temperature distribution may then be visualized with the lowest values again at the center and with the isothermal lines of similar shape to the theoretical ones but displaced with respect to them.

The average lag coefficient now assumes the place of unity in the comparison with theoretical distribution and less than average values indicate smaller lag, or higher temperatures at the location investigated, while values higher than the average indicate greater lag or lower temperature than predicted from the average relationship.

Cardioid Distribution: Assume now that at a given location the temperature is higher than the predicted one, this indicates that the lower temperature isothermal moved closer (smaller lag) to the center of the can for the time considered; for a lower actual temperature, the opposite is true and the isothermal considered is removed farther from the center. The values of assumed lag coefficients illustrate this phenomenon in Figure 8. Such an occurrence may well result in the distortion of an ellipsoidal isothermal into a cardioid one as is shown in Figure 8 for a hypothetical condition. Ultimately this results in an area of lowest temperatures off the central vertical axis as shown in Figures 6 and 7. This schematic presentation is one of many possible combinations depending on variables encountered during a thermal process.

Analysis of actual mechanism of heat penetration into a container. A separate problem is presented in attempts at determination of the causative agents behind the departure from theory other than an average correction in the theoretical relationship for the whole space of the can. In the previous discussion of the graphical representation of actual temperature distribution anisotropy, casehardening effect of chemical and

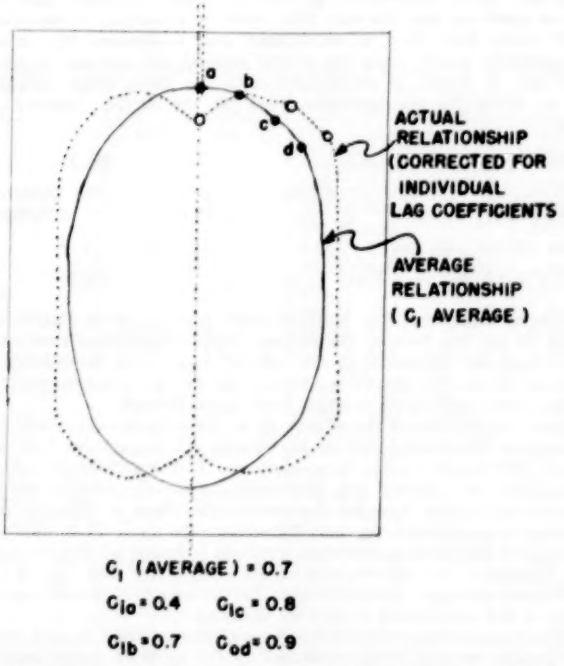

Figure 8. Hypothetical deviations from average heating equation resulting in cardioid temperature distribution.

moisture changes in meat were mentioned. In order to obtain a clearer picture of changes occurring in the container during processing, an analysis was made of the presumed actual mechanism of heat penetration compared to the ideal one. One of the causes of the actual mechanism of heat penetration being different from the ideal one is the presence of an initial temperature gradient increasing towards the peripheries of the container. Even if the uniform temperature distribution is maintained carefully before the can is put in the processing retort, the finite heating rate of the retort to the designated temperature creates a temperature gradient which causes an initial lag smaller than theoretically expected in the outside region of the can compared to the inside since obviously temperature on the periphery is higher than inside. Lengthwise, the gradient is smaller than along the horizontal plane since $(l/a) > 1$. Thus, this effect on the experimentally determined heating lag is more pronounced horizontally than vertically in terms of the computed $\hat{a}_{ii}$. The decrease in the intercept coefficient is relatively larger for the outside vertical axes than for the central vertical axis.

The average initial temperature difference (RT-IT)av. is used in the determination of $\hat{a}_{ii}$ from $\hat{a}_{ii}$ (RT-IT)av. This procedure results in use of $\hat{a}_{ii}$ which is overestimated for the center and underestimated for the outside. This effect of initial temperature gradient on computed values should be accounted for during interpretation of results based on $\hat{a}_{ii}$ values. In other words the actual heating lag in the interior of the can is smaller than indicated by $\hat{a}_{ii}$ and the reverse is true for the outside of the container. These effects are due solely to the existence of an initial non-uniform temperature distribution.

Due to fitting of only one term of the expansion of the conduction equation for heating curves, all $\hat{a}_{ii}$ are uniformly decreased. This accentuates (in terms of per cent) the previous effects when lag coefficients are calculated. The effects of anisotropy and casehardening are more pronounced for high temperatures and stronger with time because the initial distribution patterns are more disturbed, and also because more isotherms penetrate into the can. This effect of anisotropy is expressed in higher resistance to radial flow than to axial flow, and counteracts the effects of non-uniform temperatures mostly along the central vertical axis and on the vertical boundaries of the can. It results in an increase of a_{ii} on these latter boundaries and in decrease of a_{ii} along the vertical axis. Thus for the outside boundaries it can be expressed as:

$$(a_{ii}/f_s) - (\Delta_1 a_{ii}) + (\Delta_2 a_{ii}) = \hat{a}_{ii} \qquad \text{Eq. 1a}$$

(one term	)	− (non-uniform temp.	) + (anisotropy	) = (expected	)
(fitting effect)		(effect	)	(effect	)	(coefficient)

and along the vertical axis as:

$$(a_{ii}/f_s) + (\Delta_1 a_{ii})' - (\Delta_2 a_{ii})' = \hat{a}_{ii} \qquad \text{Eq. 1b}$$

The effects of departure from idealized conditions are much smaller in the intermediate space of the can because the average initial temperature used in calculations approximates the actual difference in this region. As a result, the intercept coefficients are less affected by all the factors mentioned and are more nearly equal to a_{ii}/f_s or to a_{ii} and thus may result in a greatest heating lag volume.

The relative magnitudes of the effects $\Delta_1 a_{ii}$ (due to the departure from uniform initial temperature distribution and $\Delta_2 a_{ii}$ (due to anisotropy) for both regions affect the ultimately determined $\hat{a}_{ii}$ for these locations. If the anisotropic effect were considered separately, the expected lag coefficients C_i for the central vertical axis will be lowered much more than those for the outside (the effect of fitting of one term only of the equation being assumed the same throughout the can). If in addition the effect of the presence of the initial temperature gradient is accounted for, the outcome is not certain and depends on the relative magnitudes of $(\Delta_1 a_{ii})'$ and $(\Delta_1 a_{ii})$. The resulting lag coefficients now may be lowered more for the outside than inside as was observed in the course of this experiment, or may be the same.

For certain combinations of conditions, $\hat{a}_{ii}$ may remain equal to a_{ii}/f_s or even become equal to or greater than a_{ii}. The occurrence of any of these events will depend upon the processing conditions, the size of the can and the initial temperature distribution within the can.

The assumption of 2-dimensional flow instead of the actual 3-dimensional flow could also affect the determination of intercept coefficients ($\hat{a}_{11}$). There is no way of evaluating this effect from the data collected during this investigation, although the exploration of it could prove to be of value.

RESULTS AND DISCUSSION

The values of the intercept coefficient ($\hat{a}_{11}$) anl lag coefficients ($C_1 = \hat{a}_{11}/a_{11}$) determined from the regression equations were evaluated in the search for confirmation of observed temperature distribution patterns. At the same time an attempt was made to test the applicability of the proposed analysis of mechanism of heat transfer in beef to the data obtained during the experiment, and to evaluate the relative contribution of the causative agents in distortion of theoretical temperature distributions as indicated by the determined intercept values.

a. Intercept coefficient—$\hat{a}_{11}$:

The distribution of $\hat{a}_{11}$ (Table 3) exhibited on the average the highest values for the can at locations 5, 10, 11, 12, and occasionally at locations 16, 17, and 18 (Fig. 1). The variance associated with location 5 (on the central vertical axis) was usually excessive and the student's "t"-test *(7)* did not indicate significant differences between the $\hat{a}_{11}$ values at the center and at location 5. A series of comparisons between the $\hat{a}_{11}$ value at

TABLE 2

Intercept coefficients ($\hat{a}_{11}$) for six retort temperatures determined by regression methods

Location	Retort temperature (°F.)						
	225	243	261	279	297	315	Average
1	0.732	0.907	0.829	1.560	1.575	4.091	1.619
7	0.805	0.759	1.209	0.974	1.005	1.256	1.001
8	1.008	1.235	1.320	1.564	1.101	1.552	1.297
14	0.373	1.409	1.572	1.095	1.300	1.232	1.134
20	0.735	1.145	1.670	1.523	1.106	1.594	1.296
21	0.767	0.821	0.814	0.816	1.110	1.352	0.947
22	0.454	0.997	1.056	1.130	1.229	0.917	0.964
23	0.810	1.032	1.087	0.964	1.167	0.855	0.986
24	0.611	0.903	1.165	1.059	1.322	0.855	0.986
25	0.697	0.643	0.554	0.937	1.351	0.663	0.608
2	1.069	1.334	1.622	1.348	2.241	1.541	1.526
6	0.902	1.533	1.673	1.473	1.310	1.324	1.339
9	1.075	1.401	1.886	1.613	1.708	2.141	1.637
13	1.618	1.890	1.923	1.355	2.390	1.600	1.798
15	0.775	1.442	1.605	1.294	1.112	1.209	1.240
16	1.346	2.082	1.658	1.601	1.241	1.211	1.524
17	1.267	1.363	2.230	2.109	1.521	1.261	1.625
18	1.438	1.592	1.859	1.434	1.336	1.260	1.487
19	1.288	2.123	1.456	1.449	1.231	1.304	1.475
3	1.380	1.355	1.729	1.620	1.465	1.085	1.439
4	1.463	1.3448	1.6295	1.4535	1.4453	1.2860	1.437
5	1.959	2.272	2.339	2.022	1.414	1.793	1.967
10	1.249	1.307	1.255	2.813	1.930	1.689	1.707
11	1.109	1.353	1.724	1.374	1.321	1.248	1.355
12	1.496	1.615	2.514	1.413	2.081	1.527	1.774

TABLE 3

Distribution of intercept coefficients ($\hat{a}_{11}$) in 300 x 308 can

r (cm.)	z (cm.)						
	2.75	2.00	1.00	0.00	−1.00	−2.00	−2.75
				RT = 225°F.			
0	0.732	1.069	1.380	1.464	1.959	0.902	0.805
1	1.008	1.075	1.249	1.109	1.496	1.618	0.373
2	.....	0.775	1.348	1.267	1.438	1.288	0.735
3	.....	0.767	0.454	0.810	0.611	0.697	.....
				RT = 243°F.			
0	0.907	1.334	1.355	1.345	2.272	1.533	0.759
1	1.235	1.401	1.307	1.353	1.615	1.890	1.409
2	.....	1.442	2.082	1.363	1.592	2.123	1.145
3	.....	0.821	0.997	1.032	0.903	0.643	.....
				RT = 261°F.			
0	0.829	1.622	1.729	1.629	2.339	1.673	1.209
1	1.320	1.886	1.255	1.724	2.514	1.923	1.572
2	.....	1.605	1.658	2.230	1.859	1.456	1.670
3	.....	0.814	1.056	1.087	1.165	0.554	.....
				RT = 279°F.			
0	1.580	1.348	1.620	1.453	2.022	1.473	0.974
1	1.564	1.613	2.813	1.374	1.413	1.353	1.095
2	.....	1.294	1.601	2.109	1.434	1.449	1.523
3	.....	0.816	1.130	0.964	1.059	0.937	.....
				RT = 297°F.			
0	1.575	2.241	1.465	1.445	1.414	1.310	1.005
1	1.101	1.708	1.930	1.321	2.081	2.390	1.300
2	.....	1.112	1.241	1.521	1.336	1.231	1.106
3	.....	1.110	1.229	1.167	1.322	1.351	.....
				RT = 315°F.			
0	4.091	1.541	1.085	1.286	1.793	1.324	1.256
1	1.552	2.141	1.689	1.248	1.527	1.600	1.232
2	.....	1.209	1.211	1.261	1.260	1.304	1.594
3	.....	1.352	0.917	0.855	0.853	0.663	.....

the center and the other locations with higher $\hat{a}_{11}$ values was made by means of "t"-tests of significance *(7)*. The "t" values calculated for the 1% probability level indicated the presence for every processing temperature of at least one location displaying greater heating lag than the center. Location 12 displayed heating lag greater than at the center for the retort temperatures of 243, 261, 297, and 315°F., location 5 for 225 and 279°F.; location 13 at 243 and 297°F.; and location 11 at 261 and 297°F.; location 10 at 315°F.; location 17 at 279°F.; and location 19 at 315°F. The above values are in good agreement with the temperature distributions shown on Figures 3, 4, and 5 and indicate a displacement of the location of greatest heating lag from the central vertical axis toward the outside of the container. This horizontal shift was found to be more pronounced for the middle of the temperature range used (225-315°F.) and did not confirm totally the tendency of the displacement to increase with the retort temperature.

TABLE 4
Lag coefficients ($C_1 = \hat{a}_{11}/a_{11}$) for six retort temperatures

Location	Retort temperature (°F.)						
	225	243	267	279	297	315	Average
1	0.694	0.881	0.787	1.499	1.494	3.881	1.536
7	0.764	0.720	1.147	0.924	9.954	1.192	0.950
8	0.982	1.204	1.287	1.524	1.744	1.513	1.376
14	0.364	1.373	1.532	1.067	1.267	1.201	1.134
20	0.779	1.214	1.771	1.615	1.173	1.690	1.374
21	0.666	0.713	0.707	0.708	0.964	1.174	0.822
22	0.310	0.681	0.721	0.772	0.839	0.626	0.658
23	0.515	0.656	0.691	0.613	0.742	0.544	0.627
24	0.417	0.617	0.796	0.773	0.903	0.583	0.682
25	0.605	0.558	0.481	0.813	1.173	0.576	0.701
Average	0.610	0.860	0.992	1.031	1.125	1.298	0.986
2	0.738	0.892	1.085	0.902	1.499	1.031	1.024
6	0.603	1.025	1.119	0.985	0.876	0.886	0.916
9	0.739	0.963	1.296	1.109	1.174	1.471	1.125
13	1.112	1.299	1.322	0.931	1.643	1.100	1.235
15	0.580	1.079	1.200	0.968	0.832	0.904	0.927
16	0.793	1.225	0.976	0.942	0.730	0.713	0.897
17	0.694	0.747	1.222	1.156	0.833	0.691	0.891
18	0.846	0.937	1.094	0.844	0.796	0.742	0.875
19	0.963	1.586	1.089	1.884	0.921	0.975	1.237
Average	0.785	1.084	1.156	1.080	1.033	0.946	1.014
3	0.726	0.713	0.910	0.853	0.771	0.571	0.757
4	0.717	0.659	0.798	0.712	0.708	0.630	0.704
5	1.031	1.196	1.231	1.064	0.744	0.944	1.035
10	0.676	0.707	0.679	1.521	1.044	0.913	0.923
11	0.558	0.681	0.868	0.692	0.665	0.628	0.682
12	0.809	0.373	1.360	0.764	1.125	0.826	0.960
Average	0.753	0.805	0.974	0.934	0.843	0.752	0.843
Grand Average	0.712	0.900	1.021	0.990	0.985	0.985	0.832

The existence of vertical displacement from the central horizontal plane was tested by "t"-tests between two symmetrical locations (e.g., 10 vs. 12). Significant differences (1 percent probability level) between $\hat{a}_{11}$ values for such locations were found for retort temperatures 243, 279, 297, and 315°F. There were no significant differences indicating asymmetry for the 225 and 261°F. retort temperatures. The significant differences found for the other four temperatures could not be considered as conclusive proof of asymmetry since they indicated for the same temperature simultaneously, both upward and downward shifts, depending on the pair of locations tested. It may be assumed on the basis of experimental evidence that no significant displacement from the central horizontal plane of location of greatest heating lag occurred. These results indicate that the effect of anisotropy of beef has contributed to the displacement of the location of greatest heating lag.

b. Lag coefficient—C_1:

The distribution of lag coefficients (Table 5) followed closely the distribution of intercept coefficients and gave additional evidence of the exist-

TABLE 5

Distribution of lag coefficients (C_i) in 300 x 308 can

r (cm.)	z (cm.)						
	2.75	2.00	1.00	0.00	−1.00	−2.00	−2.75
				RT = 225°F.			
0	0.694	0.738	0.726	0.717	1.031	0.603	0.734
1	0.982	0.739	0.676	0.558	0.809	1.112	0.364
2	.....	0.580	0.793	0.694	0.846	0.963	0.779
3	.....	0.666	0.310	0.515	0.417	0.605	.....
				RT = 243°F.			
0	0.861	0.892	0.713	0.658	1.196	1.025	0.720
1	1.204	0.963	0.707	0.681	0.873	1.299	1.373
2	.....	1.079	1.225	0.747	0.937	1.588	1.214
3	.....	0.713	0.681	0.656	0.617	0.558	.....
				RT = 261°F.			
0	0.787	1.085	0.910	0.798	1.231	1.119	0.147
1	1.287	1.296	0.679	0.868	1.360	1.322	1.532
2	.....	1.200	0.976	1.222	1.094	1.089	1.771
3	.....	0.707	0.721	0.691	7.96	0.481	.....
				RT = 279°F.			
0	1.499	0.902	0.853	0.712	1.064	0.985	0.924
1	1.524	1.109	1.521	0.692	0.764	0.931	1.067
2	.....	0.968	0.942	1.156	0.844	1.084	1.615
3	.....	0.708	0.772	0.613	0.723	0.813	.....
				RT = 297°F.			
0	1.494	1.499	0.771	0.707	0.774	0.876	0.954
1	1.073	1.174	1.044	0.665	1.125	1.643	1.267
2	.....	0.832	0.730	0.833	0.786	0.921	1.173
3	.....	0.964	0.839	0.742	0.903	1.173	.....
				RT = 315°F.			
0	3.881	1.031	0.571	0.630	0.944	0.886	1.192
1	1.515	1.471	0.913	0.628	0.826	1.100	1.201
2	.....	0.904	0.713	0.691	0.742	0.975	1.690
3	.....	0.906	0.702	0.296	1.213	0.423	.....

ence of doughnut-shaped volumes of lowest temperatures. It was observed, however, when comparing the values of the coefficients for the central vertical axis with those for the outside vertical axis (D-D) that for retort temperatures from 225-261°F. the outside coefficients were relatively lower than the inside ones. This occurrence indicates a relatively higher contribution of the initial temperature gradient and non-instantaneous retort come-up to the $\hat{a}_{11}$ values compared to the anisotropy effects as discussed previously. In the range of temperatures from 279-315°F. this trend has been partially reversed and the outside lag coefficients were higher than the inside ones indicating a larger contribution of the anisotropy and case-hardening effects for high processing temperatures.

The comparison of the central vertical axis (A-A) with the next closest vertical axis (B-B) indicated on the average the reality of occurrence of cardioid-shaped isothermals especially in the central portion of the

container. The lag coefficients for the central axis were lower than the coefficients for the other axes and this trend was more pronounced at higher retort temperatures.

The grouping of the values of intercept coefficients ($\hat{a}_{11}$) and lag coefficients (C_1) by locations (Tables 3 and 5) and by temperatures (Tables 2 and 4) revealed an interesting trend. The coefficients increased in magnitude with the increase in temperature up to the middle of the range tested (225-315°F.) and then decreased; however, for the outside locations the maxima were displaced towards the 297°F. retort temperature. This trend agreed well with the trends displayed by other variables tested during this investigation such as previously discussed displacement of the area of greatest heating lag, and trends for the sterilizing effect and maximum shear force values discussed elsewhere *(3)* as well as for thermal diffusivities and slopes of the heating curves *(3)*, as shown in Tables 6 and 7.

TABLE 6

Slopes (b_{11}) of heating curves and their variation

RT (°F.)	225	243	261	279	297	315	Average
$b \times 10^3$ (min.)$^{-1}$	23.75	27.29	30.27	29.58	29.62	26.24	27.79
C. V. (b_{11}) (%)	7.72	4.57	5.69	5.06	6.02	9.32	6.40
C. V. (RT-CT) (%)	33.16	18.44	21.00	16.79	17.63	11.66	19.78
C. V. (RT-IT) a_{11} (%)	10.96	5.72	7.80	5.51	6.10	4.78	6.81

TABLE 7

Average coefficients and parameters to be used with prediction equation for heating phase and their variation

RT (°F.)	225	243	261	279	297	315	Average
$R \times 10^3$ (sq. cm. /min.)	221.63	255.80	281.08	279.46	275.79	259.97	262.29
C. V. (R) (%)	7.72	4.57	5.69	5.06	6.02	9.32	6.40
C. V. ($\hat{a}_{11}$) (%)	10.46	5.86	6.60	5.47	4.56	3.28	6.37
$C_1 = \hat{a}_{11}/a_{11}$	0.708	0.896	1.020	0.992	0.990	0.896	0.934

c. Experimental errors:

The variation in the determination of the logarithm of the intercept of the heating curve (RT-IT) $\hat{a}_{11}$ (Table 6) did not affect, on the average, more than the second decimal place and thus would result in a ± 7°F. deviation. Taking into account the precision of temperature measurements (± 1°F.) it was rather small. The average coefficient of variation was found to be C. V. = 6.81%. Since each determination was taken from a regression of 9 curves, the coefficient of variation for each curve would be three times larger, which should be kept in mind when an intercept determination is made from one heating curve.

The variation of $\hat{a}_{11}$ was estimated from an approximate formula (*3*, Eq. 3) and the average coefficients of variation $\hat{a}_{11}$ are given in Table 7.

The results of the evaluation of the distribution of $\hat{a}_{11}$ and C_1 give concurrent evidence of the existence of actual temperature distributions different from the theoretically expected. The influence of the possible variation in the rate of heating at different locations in the can has not

been taken into account because of the evidence presented by the data on thermal diffusivity and "slopes" of heating curves *(3)*. These data (to be published in Part VI of this series) indicated that no significant differences were observed in the mean values of these properties which would be due to the location in the container.

SUMMARY

Experimental temperature distributions during processing of round of beef in 300-308 cans at 6 retort temperatures ranging from 225-315°F. were determined and found to be in a disagreement with theoretical expectations. The areas of greatest heating lag were found to be displaced from the geometric center of the container and to be located in a doughnut-shaped volume (torus) in the central horizontal section of the can. The form of the isothermal lines was found to be cardioid rather than eliptical in shape.

An analysis was made of causative agents for distorted temperature distributions. The reality of observed temperature distributions was confirmed by an analysis of the distribution of intercept coefficients [$a_{11} = A_{11} J_o(\mu_1 r) \sin \lambda_1 (z + l)$] of the heating equation.

On the basis of the results of this investigation it may be concluded that the temperature distributions displaying a doughnut-shaped volume of lowest temperatures in the central horizontal section of the 300 x 308 are a real occurrence caused by anisotropy of beef and non-uniform initial temperature distribution as reflected by the analysis of intercept coefficients in the heating equation.

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A NEW AND SIMPLE TECHNIQUE FOR THE DIRECT DETERMINATION OF THE EQUILIBRIUM RELATIVE HUMIDITY OF FOODS

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The development of microbes in foods may be inhibited by appropriate desiccation or by addition of solutes such as sugar or salt in sufficient quantities. The degree of desiccation or the concentration of solutes may be conveniently described in terms of *equilibrium relative humidity* of the food (symbol: h), that is: its water vapour pressure divided by the vapour pressure of pure water at the same temperature *(8, 21)*. Table 1 gives the values of equilibrium relative humidity which inhibit the development of various broad classes of micro-organisms in foods.

TABLE 1

Limiting equilibrium relative humidity (h)-values for microbes

Class	Lowest limiting h-value	References
Regular bacteria	0.90	*45, 38, 2, 3, 12, 48*
Regular yeasts	0.88	*2, 30*
Regular moulds	0.80	*41, 7, 9, 38, 8, 36*
Halophilic bacteria	≦0.75	*33, 43*
Xerophilic moulds	0.65	*9, 34, 35, 17, 12*
Osmophilic yeasts	0.61	*28, 31, 22*

Until recently the equilibrium relative humidity of a food was not easily measured, and food processing practice has been based on the water content of commodities. The water content of a food gives no explicit information on equilibrium relative humidity, however, since relatively small changes in the non-aqueous phase of a biological material (e.g. partial replacement of cellulose by sugars or starch) may strongly influence its water bonding capacity and hence its h-value at a given water content *(26)*.

It has been suggested that the equilibrium relative humidity of foods might be measured with a hair hygrometer *(19)*. Generally, however, such methods have a low reliability. When a sensitive and reliable LiCl-cell for the measurement of the dewpoint of air was announced *(11)*, an attempt was made to adapt this cell for measuring the equilibrium relative humidity of foods.

PRINCIPLES

Principle of the LiCl-cell. The essential part of the cell is a glasswool tape, impregnated with a saturated LiCl-solution. This strip is mounted on a thin-walled, lacquer-protected, stainless-steel tube. On the glasswool tape two helical silver wires are wound, between which an A.C.-potential-difference of about 25 V is maintained.

The current, passing through the LiCl-solution under the influence of the potential-difference, heats the cell. When a temperature T_h is reached where the water vapour pressure of the saturated LiCl-solution (initially of the order of 2 mm. at 20°C.) is equal to the water vapour pressure of the environment, a further increase in temperature of the cell, and therefore in its water vapour pressure, will cause evaporation of water from the saturated LiCl-solution. This leads to a residue of desiccated LiCl, possessing zero conductivity. Thereupon, heating of the cell stops and its temperature drops. At the same time water from the atmosphere is absorbed by the anhydrous LiCl. In this way the situation existing before the cell reached the temperature T_h is restored. Hence, heating occurs anew and the previous cycle is repeated, but at a greatly reduced amplitude.

Therefore, the equilibrium situation is one wherein the LiCl-cell is heated to the temperature at which the water vapour pressure of a saturated LiCl-solution (P_L) is equal to the water vapour pressure of the environment. This equilibrium temperature T_h can be measured with a mercury or a resistance thermometer. From T_h with the help of the curve, which gives the relation between the water vapour pressure of a saturated LiCl-solution and temperature, P_L can be read. This is the water vapour pressure of the environment. The relative humidity of the environment can be derived from this value in the ordinary way.

The essential advantage of the LiCl-cell-technique lies in the fact that it is a thermodynamic, rather than an empirical calibration.

Adapting the LiCl-cell to measurement of h-value. The main problem in measuring h-values of biological materials with the help of an R.H.-cell is: establishing with *sufficient rapidity* an equilibrium with the commodity under investigation *(44)*. Vacuum technique seemed at first to be helpful for this purpose, but even when an oil pump of high quality and a vacuum-tight glass measuring vat were used, unsatisfactory results were obtained, due to the very slow establishment of *temperature equilibrium* throughout the apparatus.

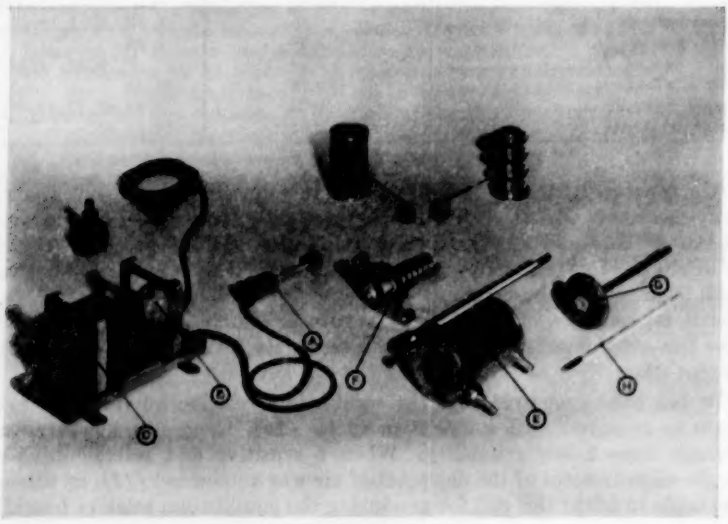

Figure 1.

It was therefore decided to use an *exposition* set up. The cell was directly exposed to coarse solid foods, contained in a coaxial cylindrical basket, made from nickel-finished brass gauze (Figure 1). For the analysis of jams, soft cheeses, doughs and allied products a set of parallel shallow dishes (Figure 1) was developed.

In order to establish a sufficient temperature control, the cell was surrounded by a coaxial, bifilar coil; the vessel, containing the substrate, was equipped with a double wall for water circulation.

CONSTRUCTION AND PREPARATION OF THE APPARATUS

The apparatus consists of:

(a) a LiCl-cell (A) with a tungsten filament lamp (B), in series with the silver wires of the cell. This lamp serves as a limiting resistor and is connected to the secondary winding of an A.C. 220/25 V transformer (C);

(b) a set of containers (D_1 and D_2) for the product under investigation;

(c) the vat (E), equipped with a permanent water circulation coil (F) coaxial with the space reserved for the cell. The coil is connected with the water circulation through the double wall of the vat.

The vat can be closed by a lid (G), provided with a hole—finished with a vapour-tight rubber joint—for the cell thermometer (H).

The LiCl-cell is cleaned by immersion in distilled water, dried, and thereupon moistened with a 5% aqueous C.P. LiCl-solution. This cleaning operation is not necessary before each set of determinations, but only when the cell is severely contaminated by a commodity previously examined.

The cell is mounted in its place in the vessel, and equipped with the cell thermometer. The transformed heating current is then applied to the cell via the lamp. When the cell thermometer shows a constant reading, the cell is in equilibrium and therefore ready for use.

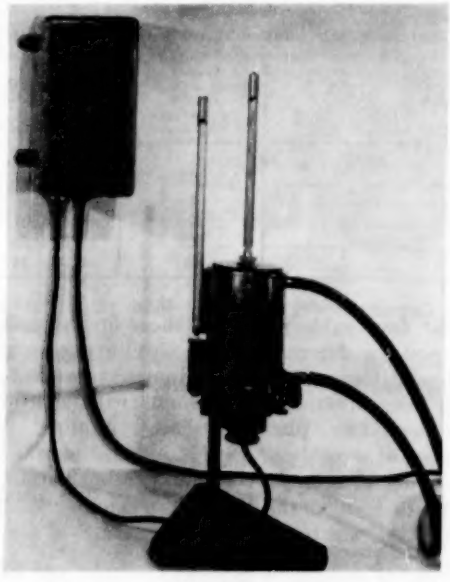

Figure 2.

PROCEDURE

The gauze basket or the set of dishes is filled with the product under investigation and then transferred to the vessel. Thereupon the top of the apparatus is closed with the lid, fitting vapour-tight around the thermometer (Figure 2).

After securing a thermostat-controlled water circulation through the wall of the vessel and the coil, the thermometer is read every 5 minutes, until variations are less than $\pm$ 0.05°C. At that moment both the cell thermometer and the vessel thermometer are read to the nearest 0.1°C.

From the reading of the cell thermometer (T_c) the water vapour pressure of the LiCl-solution (P_L) is deduced with the help of a graph (Fig. 3) giving P_L as a function of temperature. This graph was determined by the present authors by direct manometric measurement.

From the reading of the vessel thermometer (T_V) the saturation vapour pressure of pure water (P_W) under the conditions of the experiment is derived with the help of a table, giving the vapour pressure of pure water as a function of temperature, e.g. Hodgman's *(13)*.

These data enable the calculation of the equilibrium relative humidity of the substrate from the equation $h = P_L/P_W$.

An example may illustrate this type of calculation.

In the determination of the h-value of a sample of cherry jam $T_c = 59.5$°C. was read after 45 minutes; from graph 3 it was deduced that in that case $P_L = 14.8$ mm. At the time of the reading $T_V = 21.0$°C., hence from Hodgman *(13)* $P_W = 18.7$ mm. Therefore, $h = 14.8/18.7 = 0.79$.

RESULTS

The functioning of the apparatus was checked with the help of a set of reliable saturated salt solutions. The results obtained are recorded in Table 2.

TABLE 2

Saturated salt solutions used to check apparatus

Salt	Approx. sat. conc. at 20-25°C. g/100 ml *(13)*	h at 20-23°C. according. to literature *(29, 25, 39, 49)*	h_{20} found with new apparatus
K_2SO_4	12	0.97-0.99	0.97
KCl	35	0.84-0.87	0.85
NaCl	37	0.75-0.78	0.75
NH_4NO_3	270	0.64-0.67	0.64
$NaBr \cdot 2H_2O$	98	0.58-0.59	0.59
$MgCl_2 \cdot 6H_2O$	170	0.33-0.34	0.34

In view of the favourable results of these first measurements the new apparatus was used for determining the equilibrium relative humidity of a few current foods. Generally, equilibrium was reached in less than one hour. However, determinations carried out with solid foods, possessing hydrophobic surfaces (e.g. plain chocolate), required that the food be chopped into pieces of a maximum size of about 5 mm. before introduction into the cell, in order to attain a constant reading within one hour.

As the reference method the sorption technique, earlier described by the senior author *(22)*, was used. The results obtained are given in Table 3.

From the experimental data, presented in the Tables 2 and 3 it is evident that the values for equilibrium relative humidity, determined

TABLE 3

Comparison of h-values found with a classical and with the new method

Commodity	h_E determined by classical method *(22)*	h_E determined with the new apparatus
Durable sausage	0.89	0.90
Cherry jam	0.81	0.79
Orange marmalade	0.74	0.75
Breakfast cake	0.73	0.74
Whole wheat	0.71	0.72
Chocolate candy	0.69	0.69
Wheat flour	0.70	0.68
Rusk	0.32	0.33
Plain chocolate	0.31	0.32

with the new apparatus, are in excellent agreement with the data obtained in the classical, *static* method *(22)*. However, the latter method requires: (a) an equilibration period of at least a few days before reliable figures are obtained; (b) and therefore, in many cases, chemical preservation of the product under investigation. The latter is necessary in order to prevent microbial decomposition during exposure to the higher relative humidities. In other published *electric* methods 24-36 hours are required for equilibration *(44)* which period may well suffice for the occurrence of microbial activity at higher humidities.

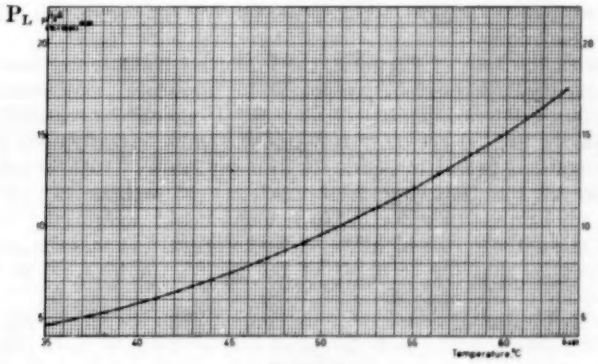

Figure 3.

The new technique is both considerably more rapid and less laborious than existing ones for the routine determination of equilibrium relative humidity.

FACTORS LIMITING BACTERIAL GROWTH IN SALTED FOODS

The efficacy of the new procedure led to a desire to apply it to salt preservation where, though it concerns one of the oldest crafts in food stabilization, fundamental data are rather scarce.

First, the equilibrium relative humidity of aqueous solutions of common salt was determined. For this purpose solutions containing 1, 3, 5, 10, 15,

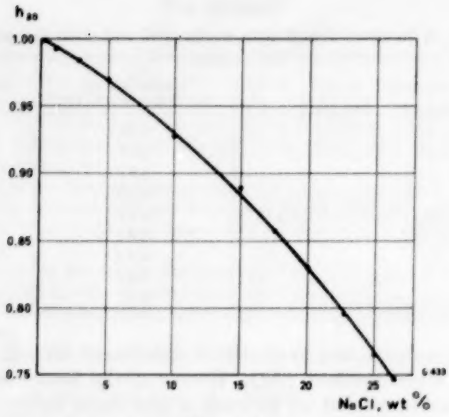

Figure 4.

18, 20 and 26.5 (saturation) weight % of C.P. NaCl in distilled water were prepared. The data obtained for the h-values of these solutions are recorded in Figure 4. Generally the data agree with those arrived at by earlier investigators *(46, 29)*, which gives further support to the reliability of the new technique.

Next, with the help of Figure 4, the inhibitive NaCl-concentrations for various food spoilage microbes, reported in literature, were recalculated to equilibrium relative humidity (Table 4). It should be realized that in doing so only a limited accuracy is obtained. Older investigators reported salt concentrations rather inaccurately in many cases; "15% of salt" may have either of the following meanings: (a) 15 weight %; (b) 15 g NaCl/100 ml brine = 15 g NaCl/110 g brine = 13.5 weight %; (c) 15 g NaCl/100 ml water = 13 weight %. From Figure 4 it may be concluded that this implies that the accuracy of the figures for h-values in Table 4 is of the order of 2% absolute.

With this limitation in mind, if we compare the limiting h-values (for the types of bacteria mentioned) in *salted media* (Table 4) with the general data, derived from measurements in partly *desiccated environments* (Table 1), the agreement is satisfactory. This suggests that in industrial salting of foods it is mainly the decrease in ERH value of the salted food which determines growth of bacteria therein.

The principle that the osmotic pressure increase caused by a solute, more than its chemical properties, governs its inhibitive effect towards microbes seems to hold true in a few more cases *(1, 20, 33, 3, 42)*, though comprehensible exceptions have been noticed as well *(9, 36, 2, 6)*.

SUMMARY

A LiCl-cell, developed for the measurement of the dewpoint of air *(11)*, has been adopted for the estimation of the *equilibrium relative humidity* of foods, the property which determines, within certain limits, the possibility of microbial spoilage.

TABLE 4

The inhibition of broad taxonomic groups of bacteria by sodium chloride

Type of organisms	Group of organisms	Authors	Inhibition by NaCl (%) = h	
Normal	Gram-negative rods			
	Mesophilic	Karaffa-Korbutt *(16)*	6-8	0.96-0.95
		Stadler *(37)*	7-10	0.95-0.93
		Horowitz-Wlassowa *(15)*	10	0.93
		Pettersson *(27)*	10	0.93
	Psychrophilic	White *(47)*	5-10	0.97-0.93
		Long and Hammer *(18)*	6-10	0.96-0.93
		Henneberg und Kniefall *(10)*	8	0.95
	Lactobacteriaceae			
	Cocci	Henneberg und Kniefall *(10)*	4->10	0.98-<0.93
		Niven *(23)*	>10	<0.93
	Rods	Niven *(23)*	>6.5	<0.96
		Hof *(14)*	7-10	0.95-0.93
		Henneberg und Kniefall *(10)*	8-15	0.95-0.88
	Spore-bearing types			
	Aerobic	Curran *(4)*	5.5	0.96
		Karaffa-Korbutt *(16)*	9-12	0.94-0.91
		Pettersson *(27)*	10-15	0.93-0.88
		Horowitz-Wlassowa *(15)*	10-16	0.93-0.87
		Schönberg *(32)*	≦15	≧0.88
	Anaerobic	van Ermengem *(5)*	6	0.96
		Pettersson *(27)*	10	0.93
		Tanner *(40)*	≦12	≧0.91
Salt-tolerant or halophilic	Staphylococci	Karaffa-Korbutt *(16)*	8	0.95
		Pettersson *(27)*	>15	<0.88
		Nunheimer *(24)*	15-20	0.88-0.83
	Micrococci	Henneberg und Kniefall *(10)*	8->15	0.95-<0.88
		Pettersson *(27)*	>15	<0.88
		Horowitz-Wlassowa *(15)*	20->25	0.83-<0.77

The products under investigation were contained in either a gauze basket (in the case of coarse, solid foods only) or in a set of parallel, shallow dishes, both coaxial with the LiCl-cell. Cell and containers were immersed in a closed metal vat, equipped with a thermostat-controlled water circulation device. In order to increase the rate of establishment of temperature equilibrium, the LiCl-cell was moreover equipped with a bifilar coil, in series with the water circulation through the wall of the vat.

Values for equilibrium relative humidity obtained in this way for foods and for sodium chloride solutions correlated satisfactorily with the data derived from measurements according to classical techniques.

Determinations according to the new method require considerably less time: one hour at the most, versus at least one day in other electric hygrometers *(44)* or several days by the older static methods *(22)*.

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STUDIES IN THE COLD STERILIZATION OF LIQUID FOODS USING MERCURY RESONANCE RADIATION. I. MILK[a, b, c]

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Common experience in the exposure of foods to ionizing radiation has been the production of certain side effects not as yet fully defined. These effects make the product unacceptable by normal consumer standards. Since less energy is developed and applied in mercury resonance radiation, it seemed that a study of the possible application of a mercury-in-quartz rare gas resonance discharge tube might provide some basic information concerning the possibilities of sterilizing liquid foods without the adverse flavor side effects heretofore encountered in high energy ionizing radiations. The scope of this paper is limited to investigations of variables directly involved in the irradiation of milk and does not encompass other liquid foods.

The desire to sterilize milk and certain milk products without adverse flavor effects has existed in the dairy industry for several decades. The commercial advantages would reside in such areas as lower materials handling costs, vastly improved storage life, enhanced acceptability, and reduction of packaging costs—to mention the more obvious ones.

It was recognized at the outset of this study that resonance radiation processing would require a supplementary heat treatment to inactivate enzymes. It has been shown that these important biological components of foods are not inactivated by commercially feasible high energy radiation processing techniques; it is true that, in some cases, in pure form, enzymes have been inactivated.

Since it has been established that sterilization of milk can be achieved with ultraviolet radiation, the problems of off-flavor development have been the primary concern of most investigators. Accordingly, a rather extensive review of this phase of the subject matter was undertaken in this study. A condensed version of this review is contained in the section that follows.

Background. The biological effects of electromagnetic radiation are usually attributed, directly or indirectly, to one of two causes: (a) con-

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[c] Presented in part (chemical techniques) before the Chicago Section of the Institute of Food Technologists, March 14, 1955, and in full before the Fifteenth Annual Meeting of the Institute of Food Technologists, Columbus, Ohio, June 15, 1955.

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version of radiant energy to heat with rise in temperature of the irradiated specimen or (b) primary or secondary chemical effects arising from the interaction of radiant energy with matter. Examples of electromagnetic radiations which result in a conversion of radiant energy to heat are infrared radiations, sonic and supersonic vibrations, and radio waves utilized in the dielectric, induction and radar heating of foods. Ultraviolet radiations, gamma rays and cathode rays result in primary and secondary chemical effects due to their interaction with matter.

Thermal effects are particularly important in explaining the biological action of radiation of comparatively long wavelengths. They become of progressively less consequence as shorter wavelengths are utilized. Chemical effects occur to some extent in the visible region but are more generally characteristic of shorter wavelengths, beginning particularly with the far ultraviolet region.

General theories and mechanisms of resonance radiation. Our primary concern is with ultraviolet radiation and with photobiological phenomena attributable to photochemical effects that arise from the excitation of molecules. Resonance radiation involves the transition of an atom from the lowest excited state to the normal state. In other words, it is the transition from a state which is achieved by direct absorption from the ground state. It should be made clear that resonance radiations are not unique to ultraviolet regions of the spectrum. For purposes of this report, however, we shall confine our interests to wavelengths less than 3000 Å.

The majority of substances that undergo or exhibit appreciable absorption in the far ultraviolet region (3000-1900 Å) undergo photochemical change in this region. At wavelengths longer than roughly 2300 Å marked absorption is exhibited primarily by organic compounds containing unsaturated groupings. In particular, it is exhibited by compounds containing conjugated double bonds. Among such compounds are the aromatic amino acids, tyrosine, tryptophane and phenylalanine, the purines and pyrimidines, unsaturated fatty acids, unsaturated steroids, carotinoids and other plant pigments, and numerous enzymes, coenzymes, vitamins and other biochemically important substances. Saturated compounds, such as simple sugars, exhibit very little absorption at wavelengths longer than about 2300 Å, and are comparatively stable throughout most of the near and far ultraviolet regions, high energies being required to produce such photochemical changes as are observed. Saturated organic compounds absorb markedly in the extreme ultraviolet region and would be expected to be quite labile in this range of high-energy quanta.

Proteins. Loofbourow *(24)* and others have shown conclusively that almost all of the absorption of proteins at wavelengths longer than about 2300 Å is attributable to the aromatic amino acids. Since the absorption of non-aromatic amino acids is inappreciable at wavelengths longer than 2400 Å, they would be expected to be relatively little affected in this range, and this had been found to be the case. From studies of dissociation spectra, the wavelength threshold for dissociation of glycine in neutral or acid solution appears to be about 1950 Å and in alkaline solution about 2200 Å *(3)*. Prolonged irradiation of glycine with ultraviolet light of sufficiently short wavelength leads to several decomposition and side reac-

tions, including hydrolysis with release of ammonia *(50)*. The foregoing results are typical and indicate that non-aromatic amino acids probably play only a small part in biological effects at wavelengths longer than about 2200 Å.

Aromatic amino acids exhibit characteristic absorption in the far ultraviolet region and are correspondingly labile. Tyrosine and tryptophane are decomposed throughout the entire far ultraviolet region and phenylalanine through at least a considerable portion of it. Typical changes involve the evolution of ammonia and an initial shift of the absorption spectrum to somewhat longer wavelengths *(2)*. Tryptophane and tyrosine take up oxygen when irradiated with far ultraviolet radiation. This is said to be the principal reason for the oxygen uptake by irradiated plasma *(15)*.

Proteins were among the first biologically important substances to be investigated photochemically, and the action of the far ultraviolet on cells was attributed very early to denaturation of proteins. Early studies *(5)* were carried out with albumin, and typical studies showed that protein exposure to far ultraviolet radiations at room temperature resulted in coagulation and development of a strong odor. When irradiated at zero degrees centigrade, the solutions remained clear until heated a few degrees subsequent to irradiation, whereupon coagulation occurred. Thus, there appeared to be a primary photochemical process, independent of temperature, and a secondary, thermally dependent process, resulting in coagulation.

In the far ultraviolet range, nucleic acid absorption is analogous to that of proteins in that substantially all of the absorption is accounted for by the unsaturated heterocyclic ring structures. That is to say, nucleic acid absorption is attributable primarily to purines and pyrimidines (uracil, guanine, adenine, thymine, and cytosine), whereas the sugars and phosphoric acid contribute negligible absorption.

Enzymes. Loofbourow *(24)* states that far ultraviolet radiation has been found to inactivate numerous coenzymes and enzymes, including catalase and lipase. The wavelength threshold at which photoinactivation begins has been studied *(8, 36)* for many enzymes, and in most instances has been found to lie in the range from about 3200 to 2900 Å. The early literature on the effects of radiation on enzymes has been summarized by Schomer *(32)*. While the above references indicate that many semipurified enzymes can be inactivated by far ultraviolet radiation, exposure times required to accomplish such inactivation as well as other physical factors involved do not lend themselves to sterilization of milk without serious deleterious effects on flavor. For example, Stassano and Lematte *(35)* in a series of experiments showed that although bacterial life in milk was quickly destroyed by ultraviolet rays, the substances contained in them, such as agglutinins, toxins and enzymes were not destroyed thereby.

Bacteria. Biologists have long recognized ultraviolet radiation as a lethal agent for the destruction of bacteria. It was early shown that such radiations exert a highly toxic influence on bacterial protoplasm exposed to them.

The application of ultraviolet radiation for sterilization or the reduction of bacterial contamination of various substances is limited, however,

by the low penetrating power of the active radiation. The common gases of the air, water vapor, substances in solution, colloidal suspensions, and obviously solids, exert a general or specific screening on the active rays. Thus, such bacterial reduction as may be accomplished by spectral energy of this nature must take place at or near the surface of the substance. Accordingly, one of the essentials in any problem of adaptation of this lethal principle is to provide surface exposure. The inherent principles operative in the mechanics of flowing fluid films of certain types, present a degree of surface exposure warranting studies designed to determine the utility and limitations of ultraviolet energy for the destruction of bacteria in milk.

In 1941, Supplee *et al. (37)* made one of the most important comprehensive studies on the effects of film thickness, flow rate and supplementary thermal processing on the treatment of milk with ultraviolet radiations from various sources. The mercury arcs used varied from one which had a strong emission at 2537 Å (85 to 94% of total output) to one which emitted no 2537 Å light, but which gave off strong radiation in the 2200 to 2300 Å region (80% of the total energy output). As a result of these studies, the authors showed (a) that a higher intensity of radiation is transmitted by milk films when subjected to 2537 Å light than when treated with light of shorter wavelengths; (b) that shorter wavelengths show a relatively inefficient bactericidal effect at lower film capacities (expressed as ounces per inch per minute), but are unexpectedly highly efficient at the larger film capacities; (c) that irradiation of stationary milk films of 0.02 ml. thickness actually appears to stimulate bacterial growth after a certain exposure time has elapsed. The U-shaped curve obtained in stationary milk-film studies appears to support in principle the observations of Coblentz and Fulton *(7)* and Hollaender *et al. (20)*. They have reported evidence of a bacteria stimulating effect resulting from sub-lethal doses of monochromatic ultraviolet radiation.

Short wavelengths are absorbed at the free interface of a flowing milk film to a much greater degree than longer wavelengths. Supplee also showed that the effectiveness of lethal ultraviolet radiation was relatively greater per unit volume of milk when applied to flowing films than when applied to stationary films having the thickness of an adhesion layer only. Supplee pasteurized milk at temperatures ranging from 162-195°F. and obtained bacterial reductions of 96 to 99.9%. These same heat-treated milks, cooled and subsequently subjected to ultraviolet light having a predominant wavelength of 2537 Å showed a slight but measurably consistent further reduction in bacteria count. The efficiency of this radiant energy treatment fell off sharply on samples which had been pasteurized at temperatures higher than 185°F. These relationships and comparisons obviously present stimulating problems of a strictly bacteriological character, as it is apparent that certain species or individual organisms inherent in the normal milk flora are not completely eliminated by this integrated application of thermal and radiant energy.

The factors of film thickness and distance of emission source from sample to be irradiated have also been given consideration by various workers. Supplee and Dorcas *(41)*, in an extensive study of transmission

of ultraviolet light having wavelengths between 2500 and 2850 Å, stated that only 20 to 40% of such ultraviolet radiations striking the surface of the milk at right angles are transmitted by films of 0.02 mm. thick. Milk films 0.11 mm. thick transmit 5% or less of the incident radiation between these limits. In the same series of experiments, it was shown that as the fat content of the milk is increased, the ultraviolet transmitting properties are decreased somewhat but not in proportion to the amount of fat present.

Supplee *et al. (38)* find that ultraviolet transmission is progressively decreased (a) by preheating to 180°F., (b) by concentrating and (c) by homogenizing as a result of physical changes in the inherent milk constituents.

Off-flavors in irradiated milk. Early attempts to irradiate milk were usually marked by a tendency to over-irradiate it with resultant deterioration in odor and taste. Schultz *(33)* attributed this to the action of hydrogen peroxide upon the protein and found that this change did not parallel the production of antirachitic potency. The irradiation of the cream alone did not affect the taste. Energy between 2600 and 3100 Å appeared less active in flavor production than that of wavelengths shorter than 2600 Å.

Although much work has been done to explain the mechanisms by which off-flavors are produced in milk, the problem still remains unsolved as to exactly what is responsible for these undesirable effects. Two distinctly different types of off-flavors have been observed in milk. It has been demonstrated that the wavelengths of light in the visible portion of the spectrum are responsible for "sunlight flavors" in milk, which have also been variously described as "tallowy," "cardboard," and "oxidized" *(10, 12)*. When milk is unduly exposed to radiation such as may be emitted by ultraviolet arcs, a flavor referred to as "activated flavor" may result, according to Weckel *et al. (47)*. These two distinctly different types of off-flavors have been studied thoroughly.

Thurston *et al. (43)* and Guthrie *(14)* attempted to correlate "oxidized," "sunlight" and "metallic" flavors with the fat content of milk. However, the report of Henderson and Roadhouse *(17)* postulated that lecithin, rather than milkfat, is the constituent affected when "oxidized" flavors develop. The fact that fat from milks exhibiting "oxidized" flavors was shown to pass only a fraction of its normal oxygen-uptake induction periods suggests that the oxidation of the milkfat does not proceed far enough to impart an "oxidized" flavor according to Henderson and Roadhouse *(17)*. Earlier work established the fact that lecithin purified from dry buttermilk (and incidentally oxidized during its purification) imparts a typical oxidized flavor to skimmilk when dispersed in the latter. This would appear to confirm the fact that it is the constituent first affected when oxidation occurs rather than the milkfat itself.

Dahle *(9)*, in a review of "sunlight" flavors, points out that the first mention of fluid milk with a "tallowy" flavor was in 1920. This flavor was readily produced by allowing milk to stand in a glass container exposed to sunlight for a comparatively short period. It was prevalent in ice cream and skimmilk treated in the same manner. It was also observed that milk developed a "cardboard" flavor when exposed to sunlight. This has been confirmed by other investigators *(44)*. Davies *(10)* reported the occurrence

of this type of off-flavor when milk was exposed to artificial light or ultraviolet light.

That metals play an important role in prdoucing this flavor defect has been shown by Roadhouse and Richardson *(30)* and others *(17)*. The relation of the presence of bacteria in milk to the possible inhibition of off-flavors was studied by Mattick *(26)* and others *(23, 25, 29)*. Other attempts to prevent formation of "oxidized" flavors involved additions of ascorbic acid *(22, 6)* and elimination of wavelengths of light shorter than 6200 Å *(21)*.

Incident to the production of "sunlight" flavors, most of the ascorbic acid and a substantial part of the riboflavin in the milk are destroyed *(19, 21)*. Sulfur-containing compounds are associated with the flavor and a derived protein from heated whey will produce the flavor on exposure to sunlight *(12, 49)*. Patton and Josephson *(28)* felt that the flavor substance has its origin in methionine and that flavor production is dependent, in large measure, on the presence of riboflavin. Sunlight was allowed to pass through flint glass bottles at 400 and 600 Weston units. It seemed of interest to determine whether other sulfur-containing amino acids, such as cysteine or cystine, would augment "sunlight" flavor in skimmilk in the manner shown by methionine. These two amino acids and methionine were added separately to quart samples of skim milk at the concentration of 20 mg. per quart. The samples, together with a control containing no added amino acids, were exposed and then examined for flavor. The samples containing cystine and cysteine exhibited the same degree of "sunlight" flavor as the control. The flavor was greatly intensified in the sample containing methionine. Thus the flavor appears to result rather specifically from the photolysis of methionine.

Dahle and Palmer *(9)* in their studies upon the formation of "oxidized" flavors found that the factor responsible for the development of the off-flavor is not necessarily responsible for the reduction of the Vitamin C content of the milk. Greenbank and Holm *(13)* pointed out that nitrogen deaeration of milk does not eliminate the formation of "oxidized" flavors inasmuch as free oxygen is not essential to milkfat oxidation since the oxygen may come from loosely bound radicals containing oxygen.

Activated flavors. Many investigators have attempted to reduce or avoid "activated" flavors (those flavors produced by ultraviolet radiations) by carefully controlling certain variables in the ultraviolet irradiation of milk. Anderson and Triebold *(2)* passed milk thruogh a commercial irradiator eight times and could detect no marked changes in the chemical composition or in the fat constants.

From an engineering point of view, the irradiation process has been studied most thoroughly by Supplee and co-workers *(37-41)*. The most significant procedure which has emerged under commercial conditions (treatment of 1000 lb. lots) is that the time of irradiation by either a quartz mercury-vapor lamp or by a carbon arc need be but a few seconds, obviating changes in taste *(39, 40)*.

The "activated" flavor in its incipient stage may be best described as "flat" and in advanced stages of development as "burnt" or "burnt protein." This flavor has a distinguishing characteristic in that it may

become more noticeable when the milk is heated to 82°C. or higher, and subsequently cooled.

Weckel and Jackson *(46)* established that milkfat was not a contributory factor to "activated" flavors but instead that these flavors were closely associated with the protein fraction of milk. Flake *et al. (12)* in a series of ultraviolet irradiation studies found no significant breakdown of proteins, although it should be pointed out that this series of experiments involved relatively mild radiation dosages. The possible role of sulfur compounds, especially methionine, as the cause of the "activated" flavors has been suggested by other workers *(48)* and irradiation is known to affect the sulfur-containing proteins usually forming sulfhydryl compounds.

Because of the confusing terminology and apparent similarities reported in connection with the production of off-flavors in milk, it is desirable to summarize the more recent theories regarding these flavors. "Oxidized" or "sunlight" flavors in milk are produced either spontaneously or as a direct result of exposure to visible light, having wavelengths longer than 3000 Å. These flavors are attributed to the oxidation of lipids. Josephson and co-workers *(21, 28)* feel that these "sunlight" flavors are due to the oxidation of lipids in conjunction with the photolysis of the amino acid, methionine. The "activated" flavors, however, are those which are produced by ultraviolet irradiation, and are believed to be due to the photolysis of the protein fraction of milk. It is doubtful that the fat portion of the milk is responsible for the production of "activated" flavors, although these flavors, once formed, may be absorbed by the butterfat of the milk. Thus it is postulated that "sunlight" flavors are produced as a result of oxidation of lipids possibly in conjunction with the photolysis of methionine, while "activated" flavors are definitely related to photolysis of the protein fraction of milk, with little or no relation to the fat portion.

EXPERIMENTAL

For a source of ultraviolet radiation, the dichromatic mercury-in-quartz rare gas resonance discharge was chosen. For these lamps, significant intensities at 1849 Å require that the lamp envelope be fabricated of a high grade of optical quartz. A source which meets the aforementioned specifications is the Hanovia "Biosteritron" lamp.

The spectral distribution of the Biosteritron is shown in Figure 1, which compares the energy distributions of the Biosteritron and a UA-2 mercury vapor arc in quartz, which is similar to the lamps used by earlier workers. It is apparent from Figure 1 that while the arc source emits only 2% of arc input at 2537 Å and virtually none at 1849 Å, the Biosteritron emits approximately 25% of arc input at 2537 Å and 2.5% at 1849 Å.

The cell, which was chosen for use in this series of irradiation studies, is made by the Nieder Fused Quartz Company and is constructed of optical quality fused quartz, with inside and outside surfaces ground to a high degree of planeness in order to avoid optical distortion. The critical dimensions of this cell are given in Figure 2.

With the exception of the above-mentioned specialized pieces of equipment, only standard laboratory apparatus and electrical equipment was utilized in the series of experiments to be described herein.

It was recognized at the inception of this work that careful control of certain critical variables would be essential for obtaining reproducible data and accordingly it was decided to hold several of the incidental factors constant throughout the series of experiments insofar as was possible.

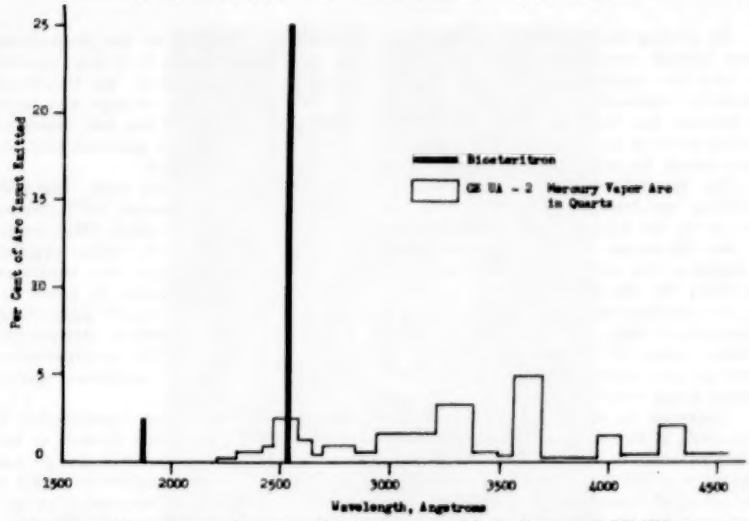

Figure 1. Comparison of energy distributions for biosteritron and GE UA-2 mercury vapor arc in quartz.

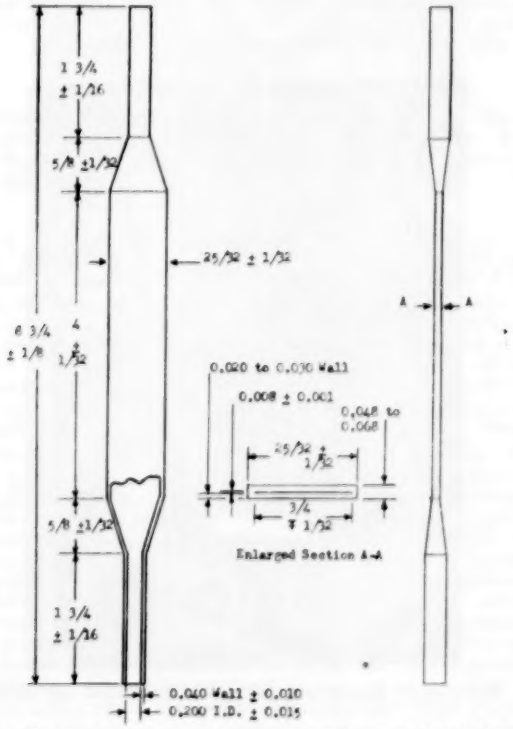

Figure 2. Nieder Fused Quartz company fused quartz irradiation cell.

By placing the fused quartz cell directly between the two grids of the Biosteritron lamp (which were 0.25 inches apart) as shown in Figures 3 and 4, it was possible to hold the distance between the emission source and both sides of the irradiated substance substantially constant (approximately 0.13 inches). No attempt was made to measure this distance and maintain precise central disposition of the cell. Since all studies were to be made using the same type of quartz cell, it was assumed that the thickness of the milk film was essentially constant (0.20 ± 0.025 mm.).

The raw milk supply, for the most part, consisted of fresh mixed milk. The milk varied in age from 10 to 30 hours and was maintained at approximately 38°F. during storage at the dairy; it was obtained as needed and used within 4 hours after arrival at the laboratory. During its subsequent storage at the laboratory while awaiting processing, the milk was maintained at 35°F. Several experiments were conducted involving the effects of radiation on raw milk which was no more than 12 hours old. A few experiments were conducted using random samples of commercially pasteurized, homogenized milk. Normal variations in raw milk made it impractical to attempt any further control of the raw milk supply. In all cases, the milks used in all experiments were sampled organoleptically to check their comparative fresh and wholesome quality before being irradiated.

Inasmuch as flavors of irradiated milk were to be given prime consideration in this work, it was necessary to verify certain results reported by earlier workers in this field. In order to confirm that methionine may be a contributing factor in the production of so-called "activated" flavors *(6, 12)*, 20 mg. of this amino acid were added to one quart of commercially pasteurized, homogenized milk picked at random. This milk was subsequently irradiated at a volume flow rate of 0.455 ml. per second. The input energy to the lamp was 75 watts. A control sample containing no added methionine was also irradiated under the same conditions.

A 16-inch electric fan was mounted in front of the Biosteritron assembly for purposes of keeping the air surrounding the lamp and cell in constant motion to avoid absorption of 2537 Å light by ozone formed during irradiation.

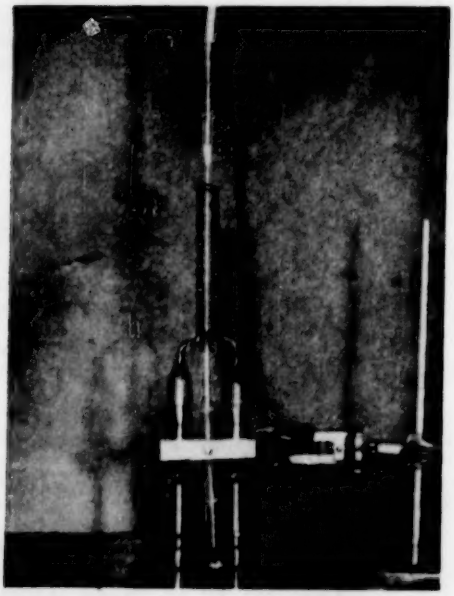

Figure 3. Mounting of biosteritron and quartz irradiation cell containing milk (end view).

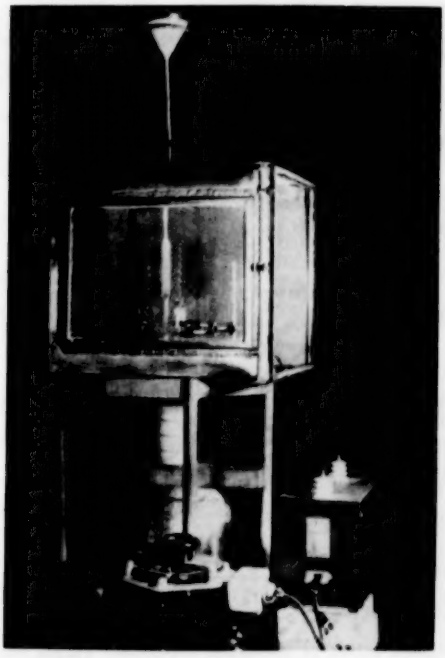

Figure 4. Milk irradiation assembly. Shown in the foreground (from left to right) are the constant voltage regulator, powerstat and 5000-volt transformer. A metal cabinet (shown above) was used to house the lamp and cell in order to minimize exposure of the operator to the ultraviolet rays.

The standard bacteriological plating procedures of the American Public Health Association *(34)* were followed as directed. Tryptone-glucose extract agar was used as the plating medium, with the prescribed addition of skim milk for dilutions greater than 1:10. The recommended incubation time-temperature relationship of 98°F. for 48 hours was adhered to, and aseptic technique was used for plating unless stated otherwise. All possible precautions were taken to prevent contamination from the surroundings; the plating area was thoroughly swabbed with a 2% solution of phenol prior to each plating operation, and sterile bacteriological equipment was used as specified above. Even with these precautions, it was impossible to prevent occasional mold contaminants from invalidating certain sets of experimental irradiation data.

Figure 4 depicts the general arrangement of apparatus used for virtually all of the experiments in this study. In order to increase contact or exposure time of the milk in the cell to the radiant energy being applied, samples were collected in sterile bottles and flasks (not shown in Figure 4); a portion of each sample was removed, chilled in ice water, and stored in a refrigerator (approximately 40°F.) for subsequent flavor and bacterial examinations. The remaining larger portion of the treated sample was then run through the cell again under identical conditions of temperature, volume flow rate and intensity of radiation, after having been previously chilled in an ice water bath inasmuch as the heat emitted by the Biosteritron lamp usually raised the temperature of the irradiated milk to about 65°F. Again, a portion of this twice-treated sample was set aside for subsequent bacterial plating and quality inspection, while the remainder was processed a third time.

When possible, milk samples were plated out immediately after irradiation. How-

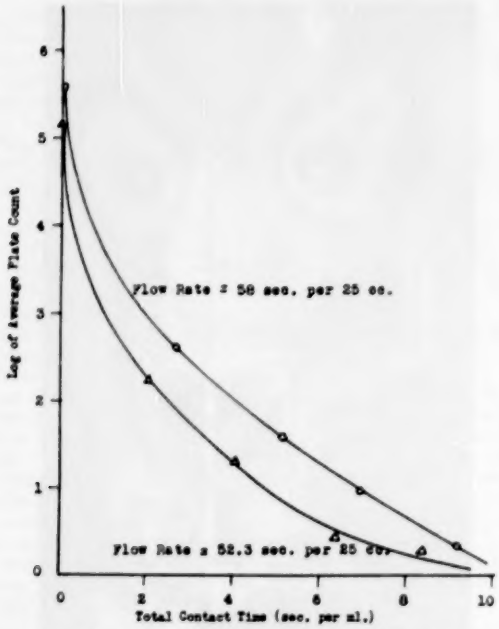

Figure 5. Bactericidal action of resonance radiations. (Input energy = 75 watts.)

ever, at no time were irradiated samples allowed to stand more than one-half hour before plating; if brief storage was necessary, the samples were incubated in a deep freeze unit which was maintained at 0°F.

RESULTS

One of the most important questions involved in this work was whether or not resonance radiations could effectively sterilize milk. Using the optimum conditions of time and intensity for such radiations as had been previously determined, the bactericidal and flavor effects of resonance radiation using an incoming milk temperature of 40°F. and an input energy of 75 watts to the Biosteritron lamp are recorded in Tables 1 and 2 and plotted in Figure 5.

By making use of a Powerstat which was incorporated in the system, it was possible to vary the input energy (i.e. watts) to the Biosteritron and hence to observe the qualitative effects of variations of lamp output on bactericidal action. Table 3 contains a compilation of representative data obtained from a series of experiments in which the same raw milk was irradiated at different intensities but at a constant incident temperature, namely 40°F.

In a preliminary experiment, sodium ascorbate was added to raw milk in a concentration of 2.23 mg. per gram milk which upon irradiation resulted in a product remarkably free of "activated" flavor but the bacterial count apparently was not appreciably affected. Therefore, the experiment was repeated using samples of raw and pasteurized milk to which had been added 160 mg. of ascorbic acid (equivalent) per gallon of milk which were then irradiated utilizing the optimum conditions as set forth above. This concentration of ascorbic acid was selected on the basis of its known effectiveness in certain thermal milk sterilization processes under study. The data shown in Table 4 summarize the results obtained with the irradiation of milk containing such ascorbic acid additions.

TABLE 1

Bactericidal and flavor effects of resonance radiation on raw whole milk. (Input energy, 75 watts; incoming sample temp., 40°F.; flow rate, 0.434 ml. per sec.)

Sample	Total contact time sec./ml.	Average standard plate count	Log. avg. standard plate count	Flavor[2]
Raw milk control	0	379,000	5.579	Clean, creamy
Raw milk—irrad.	2.31	435	2.638	Tr. act.
Raw milk—irrad.	4.62	38	1.580	Tr. act.
Raw milk—irrad.	6.93	8[1]	0.903	V. sl. act.
Raw milk—irrad.	9.24	2[1]	0.301	V. sl. act.

[1] Statistically unreliable plate count (less than 30 colonies per plate).
[2] In all subsequent flavor and sample notations, the following system of abbreviations will be adhered to: AA—ascorbic acid added; act.—"activated"; ast. aft.—astringent aftertaste; def.—definite; irrad.—irradiated; mod.—moderate; sl.—slight; temp.—temperature; tr.—trace; v.—very; sec.—second; ml.—milliliter.

TABLE 2

Bactericidal and flavor effects of resonance radiation on raw whole milk (input energy, 75 watts; incoming sample temp., 40°F.; flow rate, 0.488 ml. per sec.)

Sample	Total contact time sec./ml.	Average standard plate count	Log. avg. standard plate count	Flavor
Raw milk control	0	156,000	5.193	Clean, creamy
Raw milk—irrad.	2.05	180	2.255	Clean
Raw milk—irrad.	4.10	22	1.342	Tr. act.
Raw milk—irrad.	6.15	3[1]	0.477	V. sl. act.
Raw milk—irrad.	8.20	2[1]	0.301	Sl. act.
Raw milk—irrad.	10.25	0[1]	.....	Mod. act.

[1] Statistically unreliable plate count (less than 30 colonies per plate).

TABLE 3

Bactericidal action of Biosteritron vs. lamp input energy. (Initial plate count of raw milk = 51,000 org. per ml.)

% line voltage	Input energy, watts	Total contact time, sec. per ml.	Average plate count, organisms per ml.	Log of avg. plate count
40	56	2.10	110	2.041
45	70	2.10	40	1.602
55	84	2.10	27	1.431
75	98	2.10	120	2.079

A second series of experiments was conducted to observe the effects of irradiation on the ascorbic acid content of similarly fortified raw whole milk with increasing contact times, the results of which are summarized in Table 5 and illustrated graphically in Figure 6.

The various basic experiments performed with resonance radiations had been primarily concerned with bactericidal action and reduction, or practical elimination, of flavor and taste side-effects. With the exception of one or two isolated experiments, it was not possible to irradiate milk by resonance radiation as described above without encountering the formation of "activated" flavors in varying degrees. The reasons for these variations are not known at this time.

It was observed that the addition of lactose to previously irradiated milk did apparently tend to mask "activated" flavors to some degree, but it did not prevent such off-flavors being detected by persons with trained taste acuities. Earlier experiments by Dunn *et al. (11)* suggested that possibly the prior inoculation of raw whole milk with *Streptococcus lactis* cultures might sufficiently increase the initial bacterial

TABLE 4

Effects of ascorbic acid fortification upon "activated" flavor development and bactericidal action of resonance radiations applied to both raw and pasteurized milks (input energy, 75 watts; incoming sample temp., 40°F.; fortified milks containing 160 mg. ascorbic acid equivalent added per gallon of milk).

Sample	Total contact time sec./ml.	Average standard plate count	Log. avg. standard plate count	Flavor
Raw milk control	0	54,000	4.732	Bland
Raw milk—irrad.	2.28	60[1]	1.778	Tr. act.
Raw milk—irrad.	4.51	6[1]	0.778	V. sl. act.
Raw milk—irrad.	6.75	0[1]	.....	Sl. act.
Raw milk—irrad.	9.01	0[1]	.....	Mod. act.
Raw milk fortified with ascorbic acid	0	93,000	4.968	Clean, creamy
" " —irrad.	2.28	240[1]	2.380	V. sl. act.
" " —irrad.	4.48	25[1]	1.397	Sl. act.
" " —irrad.	6.73	5[1]	0.699	Mod. act.
" " —irrad.	8.98	13[1]	1.114	Mod. act.
Pasteurized milk fortified with ascorbic acid	0	30,000	4.477	Clean, creamy
" " —irrad.	2.23	185	2.267	Tr. act.
" " —irrad.	4.46	7[1]	0.845	Mod. act.
" " —irrad.	6.71	75	1.875	Mod. act.
" " —irrad.	8.92	75	1.875	Def. act.

[1] Statistically unreliable plate counts—less than 30 colonies per plate.

count of the inoculated milk to a degree whereby the Biosteritron lamp radiations might exhibit selective action on these and other bacteria present in preference to those on the proteins and other possible sources of off-flavor production in the milk. This idea seemed worth trying inasmuch as the source of resonance radiation being used in the foregoing experiments as described was known to exhibit selective action on peptide bonds *(31)*.

Accordingly, two special bacterial cultures of *Streptococcus lactis* were obtained from Christian Hansen Laboratories of Milwaukee, Wisconsin, and were identified as Hansen Strains 22 and 23, respectively. Raw milk was divided into 3 portions. One portion was set aside as a control; another aliquot was inoculated with 1 ml. of culture

TABLE 5

Effects of resonance radiations on ascorbic acid content and bacterial destruction when applied to raw whole milk fortified with 160 mg. ascorbic acid (equivalent) per gallon of milk. (Input energy, 75 watts; incoming milk temp., 40°F.)

Sample	Total contact time sec./ml.	Average standard plate count	Log. avg. standard plate count	Mg. ascorbic acid per gram of milk
Raw whole milk fortified with ascorbic acid	0	75,000	4.8751	0.0697
" " " —irrad.	2.02	520	2.7160	0.0521
" " " —irrad.	4.02	9[1]	0.9542	0.0348
" " " —irrad.	6.02	6[1]	0.7782	0.0261

[1] Statistically unreliable plate counts—less than 30 colonies per plate.

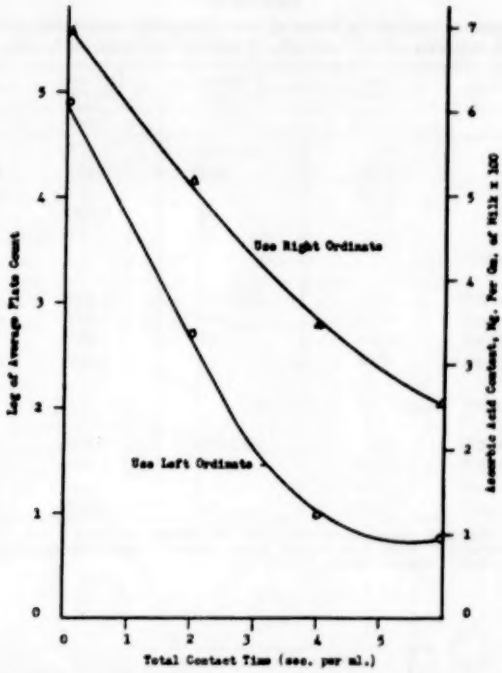

Figure 6. Effects of resonance radiation on bacterial population and ascorbic acid content of raw milk fortified with 160 mg. of ascorbic acid per gallon of milk.

No. 22 per 100 ml. of raw milk 2 hours before the milk was to be irradiated, and a third aliquot was inoculated with culture No. 23 in the same manner as described above. The inoculated samples were kept under refrigeration at 35°F. until ready to be processed. The incoming milk temperature was maintained at 40°F. for all runs, and the lamp input energy was kept constant at 75 watts. Table 6 and Figure 7 illustrate the results obtained in this experiment.

Inasmuch as it was desired to avoid the use of fermented whole milk prepared with a *Streptococcus lactis* culture in order to minimize any possible flavor effects contributed by such a milk culture, it was decided to prepare a culture in a partially deproteinized whey substrate. Accordingly, a heat-treated cottage cheese whey was clarified to remove the precipitated albumin, adjusted to pH 7.0 and inoculated with *Streptococcus lactis*, Hansen strain 22, and incubated for 16 hours at 70°F. The protein content of this heat treated whey was found to be 0.40% (N × 6.38 Kjeldahl).

The incubated whey culture of *Streptococcus lactis* upon removal from the incubator was plated in suitable dilutions using standard tryptone-glucose extract agar fortified with non-fat milk solids as prescribed by the Standard Methods of Milk Analysis to determine its bacterial plate count. Portions of this whey culture were also used to make 5% inoculations of raw whole milk and pasteurized coffee cream (so-called "half and half"). Samples of the inoculated raw whole milk and pasteurized coffee cream were also plated to determine their bacterial plate counts prior to their irradiation as well as subsequent platings to determine the bactericidal effects of their irradiation. The results are summarized in Table 7 and Figure 8.

TABLE 6

Effects of resonance radiations on flavor of raw whole milk inoculated with Streptococcus lactis milk cultures. (Input energy, 75 watts; incoming milk temp., 40° F.)

Sample	Total contact time sec./ml.	Average standard plate count	Log. avg. standard plate count	Flavor
Raw milk control	0	120,000	5.079	Clean
Raw milk—irrad.	2.31	300 [1]	2.477	Tr. act.
Raw milk—irrad.	4.62	.....	.....	Sl. act.
Raw milk—irrad.	6.93	4 [1]	0.602	Def. act.
Raw milk inoculated with *S. lactis* culture Hansen #22	0	865,000	5.937	Clean, sl. cult.[2]
" " —irrad.	2.32	300 [1]	2.477	Sl. cult., tr. act.
" " —irrad.	4.64	80 [1]	1.903	Tr.cult., sl. act.
" " —irrad.	6.96	10 [1]	1.000	Sl. act.
Raw milk inoculated with *S. lactis* culture Hansen #23	0	92,000	4.964	Sl. sour
" " —irrad.	2.32	1,000 [1]	3.000	Tr. sour
" " —irrad.	4.64	.....	.....	Sl. cult., sl. act.
" " —irrad.	6.96	25 [1]	1.398	Tr. cult., sl. act.

[1] Statistically unreliable plate counts—less than 30 colonies per plate.
[2] Culture—a typical *S. lactis* milk culture flavor, richer in taste sensation than raw whole milk with distinctive so-called "starter" flavor.

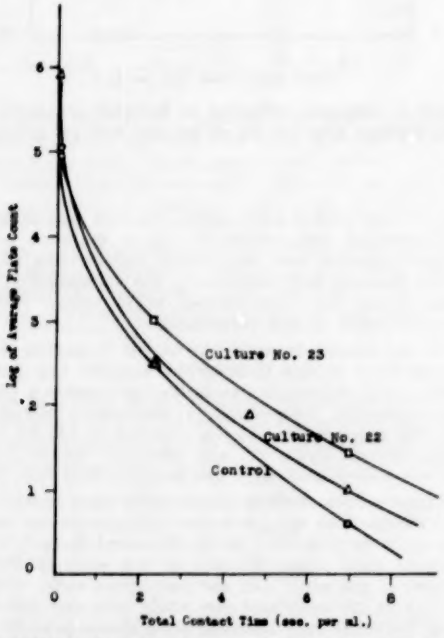

Figure 7. Bactericidal action of resonance radiation on milk inoculated with lactic acid "starter" culture (input energy = 75 watts).

TABLE 7

Bactericidal and flavor effects of resonance radiation applied to whey culture of Streptococcus lactis, Hansen strain No. 22, and to raw whole milk and pasteurized coffee cream inoculated with 5% of the deproteinized whey culture prior to irradiation. (Input energy, 75 watts; incoming sample temp., 40°F.)

Sample	Total contact time sec./ml.	Average plate count org./ml.[2]	Log. average plate count	Flavor
Partially deproteinized whey culture—				
S. lactis, Hansen #22	0	2.9×10^9	9.46	Acid, tart
" " —irrad.	1.44	90,000	4.95	Acid, tart
" " —irrad.	2.90	Less than 100[1]	2.00	Acid, tart
" " —irrad.	4.36	Less than 1[1]	0	Acid, tart
" " —irrad.	5.80	Less than ½[1]	0	Acid, sl. sweet
Raw whole milk	0	224,000	5.35	Clean
Raw whole milk plus 5% deprot. whey cult.	0	985,000	5.99	Clean, rich
" " " —irrad.	2.10	5,100	3.71	Clean
" " " —irrad.	4.20	165	2.22	Clean, tr. act.
" " " —irrad.	6.30	4[1]	0.60	Clean, sl. act.
Pasteurized cream	0	.....	.....	Clean, rich
Pasteurized cream plus 5% deprot. whey cult.	0	.....	.....	Clean, rich
" " " —irrad.	3.28	.....	.....	Sl. nutty
" " " —irrad.	6.57	.....	.....	Sl. act.
" " " —irrad.	9.85	.....	.....	Def. act.

[1] Unreliable plate count (less than 30 colonies per plate). Less than 100 means no growth in plates of 1/100 dilution. Less than 1 means no growth in plates of 1 ml. samples. Less than ½ means no growth in plates of 2 ml. samples.
[2] Plates incubated at 70°F. for 72 hours plus 48 hours at 98°F.

DISCUSSION

It was quite apparent from the irradiation of the whey culture of *Streptococcus lactis*, Hansen strain 22, that these microorganisms were killed much more rapidly and with no discernible off-flavor developments being noted even with increasing irradiating exposures when compared with the effects observed when these and similar microorganisms were subjected to resonance radiation in raw whole milks or pasteurized milk products. Obviously, the low protein content of the whey itself contributed to such results.

It was most interesting to observe that the "activated" flavor intensities in the increasing irradiation exposures of milk and cream samples inoculated with the partially deproteinized whey cultures of *Streptococcus lactis*, Hansen strain 22, were considerably reduced when compared with previous effects noted in milks inoculated with milk cultures of these and similar microorganisms; thus lending support to a theory that these microorganisms may be preferentially destroyed in their substrates, and under the proper conditions of their inoculations (population numbers) might conceivably result in virtual elimination of the development of so-called "activated" flavors in milks subjected to the resonance radiations as described.

The foregoing results, as well as the suggested theory of preferential destruction of bacteria, imply that further research could well be directed

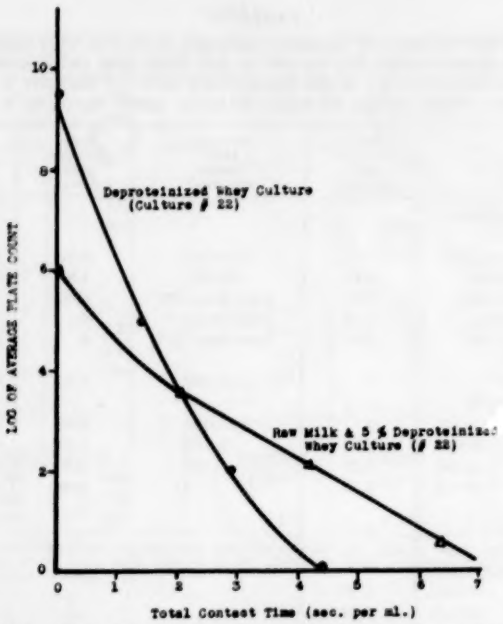

Figure 8. Bactericidal effects of resonance radiation on raw milk inoculated with deproteinized whey lactic acid culture. (75 watts, 40°F.).

towards the contributory factors apparently involved in the reduction of so-called "activated" flavor developments encountered in the resonance radiations as described.

The enhanced bactericidal effectiveness of the resonance radiations utilized in this study are attributed to at least four factors, namely: (1) the emission by the Biosteritron lamp of primarily only two wavelengths of high bactericidal efficiency, such emission consisting of approximately 90% of total lamp output at 2537 Å and about 9% at 1849 Å; (2) the absence in the Biosteritron source of frequencies of low bactericidal efficiency but high activity in bacterial stimulation; (3) the exposure of two plane surfaces of a moving film of raw whole milk of approximately 0.20 mm. thickness in a fused quartz cell which afforded maximum transmission of the two aforementioned wavelengths of light; (4) a marked reduction in effective exposure or contact time required. Obviously, the rate of flow of the film of raw milk within the cell influences the exposure or contact time of any given portion of the milk thus exposed to the radiant energy being emitted by the Biosteritron lamp.

The addition of minute amounts of methionine to raw whole milk prior to its irradiation resulted in intensifying the "activated" flavor encountered in using resonance radiations as described. However, raw whole milks containing no added methionine varied in the intensities of this off-flavor development, some milks apparently being more susceptible to such development than others.

In one or two isolated instances, no perceptible off-flavors were encountered in irradiated raw whole milks for reasons which are not apparent at this time. In view of the fact that *whole* milks were used throughout this study, it is not believed that any oxidation of milkfat is involved or responsible for the production of the "activated" flavors encountered.

In all instances of off-flavor developments in both raw whole milks and conventionally pasteurized milk products subjected to the resonance radiations applied as described, they were characterized by varying degrees of the "activated" tastes and flavors, which confirms the observations of Weckel and Jackson *(46)*.

The intensities of off-flavors produced by the resonance radiations applied as described can be substantially reduced, if not practically eliminated, by suitable inoculations of the raw milk under treatment with appropriate cultures of *Streptococcus lactis* prior to the irradiation of such previously inoculated raw milks. However, the influences contributed by such microorganisms in minimizing such off-flavor developments are variable and are not fully understood at the present time.

Fortification of raw whole milks and pasteurized, homogenized whole milks with small amounts of ascorbic acid did not appreciably affect the intensity of the "activated" flavors encountered nor did they noticeably affect bactericidal effectiveness of resonance radiations applied as described. However, there were indications that increasing the amounts of ascorbic acid could result in preferential absorption of the actinic radiation by the ascorbic acid, with concomitant decrease in bactericidal efficiency.

A temperature of approximately 40°F. provided optimum flow rate conditions in the special fused quartz cell used for maximum bactericidal effectiveness of the resonance radiations applied as described.

With the raw whole milks studied, it appears theoretically possible to achieve practically zero standard bacterial plate counts by subjecting such milks at an initial temperature of approximately 40°F. to the resonance radiations applied as described with an input energy of 75 watts and using total contact times of 10 seconds or less.

SUMMARY

The general objective of this investigation was to determine whether milk could be subjected to resonance radiations under conditions that would provide the virtual destruction of its contained microorganisms without encountering objectionable flavor side-effects. If this could be accomplished, a further objective might be to determine the economic feasibility of its contributing a substantial technological advance in the preservation of milk and its products.

From the results that have been obtained, and reported upon herein, it would appear that both these objectives are possible of eventual realization. At the very least, they suggest the advisability of further pursuing this interesting field of radiation for a more complete understanding of its effects upon such foods as it might effectively preserve. Such a pursuit promises to add to the sum total of the rapidly accumulating store of information on the effect of electromagnetic radiation in both the ionizing and non-ionizing regions.

In other words, the resonance irradiation of foods would seem to be not only a fruitful field in its own right, but such studies would provide data of value in interpreting the action of ionizing radiations upon a wide variety of irradiated foods.

Acknowledgment

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ANTIOXIDANT PROPERTIES OF SPICES IN OIL-IN-WATER EMULSIONS[a, b, c]

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It has been reported previously that virtually all samples examined of 32 varieties of spices exhibited antioxidant properties in lard, when tested by the active oxygen method (A.O.M.) at 98.6°C. *(2)*. Although most of the spices possessed only weak antioxidant activity, several were shown to be powerful antioxidants. The "carry through" antioxidant activity was also determined in baked pie crusts. Most of the spices were found to be only slightly antioxygenic, 6 were prooxygenic, and 4 prolonged the stability of pie crusts by factors of 2 to 4.5. In general, however, the effectiveness of spices in protecting baked pie crusts against rancidity was appreciably lower than their ability to protect lard.

This was in agreement with the results of others *(4, 6)* who had found that typical phenolic antioxidants were much less effective in protecting baked goods than in protecting lard. Mattil and Black *(7)* related the water solubility of an antioxidant to its ability to stabilize the fat in baked goods. Their results indicated that in baked goods where the fat containing the antioxidant is in contact with water, if the solubility of the antioxidant in water is appreciable, it may be extracted from the fat and thus be ineffective. Lehmann and Watts *(5)* reported that of five phenolic antioxidants tested in dry lard, and in lard in contact with an aqueous phase, three had approximately the same effectiveness in both substrates while one was more effective in the dry lard and one more effective in the lard-water system.

Spices are seldom used in pure fats or in pie crusts. Furthermore, antioxidants used in pie crusts are not only in contact with an aqueous phase, but are also subjected to relatively high temperatures for various lengths of time, a treatment that may well destroy much of the antioxidant. Spices, however, are used in a number of other fat-containing food products in which the fat phase is in intimate contact with an aqueous phase, and many of these products are not subjected to any heat treatment. It was thought desirable, therefore, to study the antioxidant effectiveness of spices in a two-phase aqueous-fat system. Since different spices are used in different foods, it was believed that a simple oil-in-water emulsion would best represent the basic conditions encountered in food products in which the fat is in contact with an aqueous phase. The use of such a substrate would permit the comparative evaluation of all spices in a simple system uncomplicated by the presence of other food components.

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[b] Presented at the 14th Annual Meeting of the Institute of Food Technologists, June 27-July 1, 1954, Los Angeles.

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EXPERIMENTAL

Emulsions have a tendency to be unstable at high temperatures and therefore the tests were conducted at a relatively low temperature. This in turn necessitated the use of a liquid fat with a low resistance to oxidation so that results would be obtained in a reasonable length of time.

From the standpoint of fatty acid composition, corn oil is characteristic of fats commonly used in certain food products. Corn oil, however, contains appreciable quantities of antioxidants as normal components which in this case would not only make the time required to perform the experiments excessive, but might also mask the effects of the antioxidants in the spices. Antioxidants have been removed from fats by treatment with adsorbents *(1, 3, 8)*. Therefore, corn oil from which most of the normally occurring antioxidants had been removed was used for this study.

Stability measurements were made by an oxygen absorption technique using a Warburg apparatus.

Preparation of antioxidant-free corn oil. A 25% solution of corn oil in petroleum ether (500 ml. of corn oil in 1500 ml. of Skellysolve F) was passed over a 5×22.5 cm. column of activated alumina, a 5×10 cm. column of silicic acid, a 2.5 cm. layer of charcoal between two 1 cm. layers of anhydrous sodium sulfate in an 11 cm. Buckner funnel, and finally through two more 5×22.5 cm. columns of activated alumina. All these operations were performed in an atmosphere of nitrogen. The colorless percolate was evaporated under vacuum yielding a nearly colorless oil with a peroxide value of 2.5 m.e./kg. In the A.O.M. apparatus at 98.6°C., the peroxide value of this oil rose to 25 m.e./kg. in 15 minutes, and the oil was therefore considered to be sufficiently free of antioxidants for the purposes of this study.

Preparation of emulsions. Attempts to prepare corn oil-water emulsions without an emulsifier were unsuccessful. Stable emulsions were prepared by shaking together vigorously 50 ml. of purified corn oil and 50 ml. of distilled water containing 1 ml. of Tween 20 (polyoxyethylene sorbitan monolaurate), and passing the coarse suspension five times through a small hand homogenizer. The flask containing the emulsion was then alternately evacuated and refilled with nitrogen several times to remove the occluded and dissolved oxygen. The emulsion was stored under vacuum in a refrigerator until used. Emulsions prepared and stored in this manner have been kept for periods up to three months with no evidence of separation.

Preparation of samples. About 30 mg. of ground spice was weighed in a small flask and enough of the corn oil emulsion added to give a concentration of 0.2% of spice in the whole emulsion. After mixing thoroughly, a 5 ml. aliquot of the mixture was measured into each of two cylindrical Warburg flasks approximately 60 mm. in diameter and 30 mm. high. The flasks were attached to their manometers, flushed with oxygen, and placed in the bath at 40°C. After a 10-miute equilibration period the manometers were closed and changes in manometer readings recorded as a function of time. In order to keep the spice particles in suspension in the emulsions, the flasks were shaken at the rate of 120 oscillations per minute. Each spice was tested in duplicate, and duplicate control samples consisting of 5 ml. of corn oil emulsion to which no spice had been added were included in each run.

RESULTS AND DISCUSSION

Figure 1 shows typical oxygen absorption curves for several samples of emulsion containing some of the spices. The initial rates of oxygen absorption were markedly different for different spices, varying from no measurable oxygen uptake for cloves, to 0.018 ml. per hour for mustard and 0.049 ml. per hour for the controls. When oxygen absorption was plotted against time, the slopes of the curves increased gradually and no sharp break marking the end of the induction period could be detected. Furthermore, in many cases, the changes in rates of oxygen absorption were approximately constant over long periods and exhibited no characteristics that could be interpreted as a measure of an induction period.

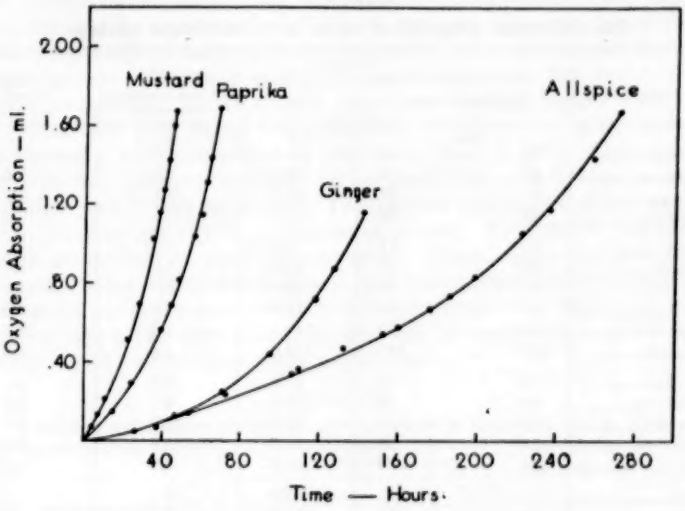

Figure 1. Oxygen absorption of emulsions containing spices.

The stability of each sample was therefore measured in terms of the time required for the sample to absorb 1 ml. of oxygen. This corresponded approximately to an oxygen absorption of 40 m.e. of oxygen per kilo of fat in the sample.

The stabilities of the emulsions are shown in Table 1. Excellent agreement was found in the oxygen absorption of duplicate samples of each control. There are some variations, however, in the stabilities of the 10 controls. These variations may well be a result of differences in the emulsion particle size and of changes taking place during storage of the emulsions and of the antioxidant-free corn oil. Duplicate values for the stabilities of the samples containing spices are also generally in good agreement. The average deviation from the means for all samples is 5%. The highest deviation from the mean (17.0% in the case of marjoram) is not excessively great when one considers that there are a number of variables involved in emulsion systems that are not encountered with simple fat substrates. Among such variables are the rate of distribution of the active compounds from the spice particles into the fat phase, the effect of agitation upon the settling of particles of different spices, difficulty in exact duplication of samples, and the effect of the spices on the physical state of the emulsion. All samples of oregano and one turmeric sample caused a visible separation of the emulsions, but it was not further determined whether this involved the action of specific substances in these spices, or purely physical effects of the solid particles.

All the spices studied were effective in protecting the emulsions against oxidation, but the order of effectiveness of various spices in the emulsion system was quite different from that observed in the systems previously studied. For most spices the antioxidant indexes obtained in emulsions

TABLE 1

The antioxidant properties of spices in an oil-in-water emulsion

Spice	Stability				Antioxidant index[1]
	Replicate values		Mean	Average deviation from mean	
	hrs.		hrs.	%	
Control	12	13	12.5	4.0	....
Aniseed	57	62	59.5	4.2	4.8
Basil	46	53	49.5	7.1	4.0
Cardamon	63	66	64.5	2.3	5.2
Control	17	17	17.0	0	....
Caraway	53	45	49.0	8.2	2.9
Celery seed	51	59	55.0	7.3	3.2
Chili	39	45	42.0	7.1	2.5
Cassia	146	150	148.0	1.4	8.7
Cinnamon	140	144	142.0	1.4	8.4
Control	15	16	15.5	3.2	....
Foenugreek	26	26	26.0	0	1.7
Mustard	37	35	36.0	2.8	2.3
Poppy	33	30	31.5	4.8	2.0
Ginger	136	134	135.0	0.7	8.7
Control	13	13	13.0	0	....
Coriander	33	33	33.0	0	2.5
Cumin	30	37	33.5	10.4	2.6
Fennel	35	35	35.0	0	2.7
Control	22	24	23.0	4.3	....
Marjoram	78	110	94.0	17.0	4.1
Black pepper	124	156	140.0	11.4	6.1
White pepper	108	122	115.0	6.1	5.0
Nutmeg	200	222	211.0	5.2	9.2
Mace	276	312	294.0	6.1	12.8
Control	18	20	19.0	5.3	....
Sage A	141	155	148.0	4.7	7.8
Sage B	123	143	133.0	7.3	7.0
Rosemary A	190	197	193.5	1.8	10.2
Rosemary B	190 228	233	217.0	8.3	11.4
Control	13	13	13.0	0	....
Paprika	51	55	53.0	3.8	4.1
Oregano[2]	96 95	117	103.0	9.4	7.9
Turmeric[2]	202 211		207.0	2.2	15.9
Turmeric	384		384.0	....	29.6
Control	15		15.0	....	....
Dill	31	33	32.0	3	2.1
Bay leaves	64	68	66.0	3	4.4
Red pepper	49	49	49.0	0	3.3
Control	15	15	15.0	0	....
Thyme	93	110	101.5	8	6.8
Savory	118		118.0	....	7.9
Allspice	220	270	250.0	12	16.7
Cloves	1060	1395	1228.0	16	81.8
Control	13	13	13	0	....
Cloves	1150	1530	1340.0	14	103.0
Control	17.8			....	....
Cloves	1290			....	72.5

[1] Ratio of mean stability of sample to mean stability of control.
[2] Emulsion unstable.

were several times greater than those observed with a simple lard substrate using the active oxygen method. Table 2 compares the antioxidant indexes, in emulsions and in lard, of those spices that were either less effective or unusually more effective in emulsions than in dry lard. The ratios of these indexes are given in the last column as a measure of the relative antioxidant activity of each spice in the two substrates. Of the spices tested, only sage and rosemary were less active in emulsions than in lard. Although they were still very good antioxidants in the emulsion system, they were only about half as effective as in lard. Foenugreek and mustard had approximately the same activity in emulsions as in lard. Foenugreek was also the weakest antioxidant in the emulsion system. Cassia, cinnamon, and allspice were rather weak antioxidants in lard, but were quite effective in emulsions. Both cassia and cinnamon were slightly prooxygenic in baked pie crusts. Turmeric was a fair antioxidant in lard and is much better in emulsions, particularly if the emulsion does not separate.

TABLE 2

Relative antioxidant activity of several spices in emulsions and in lard

Spice	Antioxidant index		Relative antioxidant activity
	Emulsion	Lard[1]	
Sage B	7.0	16.5	0.42
Sage A	7.8	14.2	0.55
Rosemary A	10.2	17.6	0.58
Rosemary B	11.4	17.6	0.65
Cassia	8.7	1.4	6.2
Cinnamon	8.4	1.3	6.5
Allspice	16.7	1.8	9.3
Turmeric	15.9[2]	2.9	5.5
Turmeric	29.6	2.9	10.2
Cloves	81.8	1.8	45.3
Cloves	103.0	1.8	57.3

[1] Active oxygen method at 98.6°C.
[2] Emulsion unstable.

The best spice in the oil-in-water emulsion system was cloves. The sample of cloves in three different emulsions gave antioxidant indexes of 72.5, 82, and 103, approximately 50 times as large as that obtained with lard alone. The antioxidant power of cloves in an oil-in-water emulsion has also been determined by measuring peroxide accumulation during storage at 37°C. The peroxide value/time curves were autocatalytic and the rate of peroxide accumulation increased rapidly between peroxide values of 15 and 25 m.e./kg. Peroxide values of 20 m.e./kg. were attained by the controls in 3 hours and by the samples in 167 and 197 hours (average 182 hours). The average antioxidant index calculated from these values is 60.

When tested in lard, rosemary and sage were the best antioxidants of all the spices studied and their activities were approximately equal. In pie crusts, however, rosemary was definitely superior to sage; in the emulsion studies rosemary was also definitely superior to sage. In addition to cassia, cinnamon, allspice, turmeric, and cloves, it was found that ginger, mace, nutmeg, and oregano were also better than sage; black and white peppers were nearly equal to sage in protecting emulsions. All these spices were much inferior to sage when studied in lard.

SUMMARY

The antioxidant properties of 32 spices have been studied in a simple oil-in-water emulsion system. All spices protected the emulsion against oxygen absorption. In most cases the spices were more effective in the emulsions than in plain lard or baked pie crusts, but the order of effectiveness was different for the different substrates. Cloves were extremely effective in preventing oxidation of the emulsions. Other spices with antioxidant indexes greater than 5.0 were allspice, cardamon, cassia, cinnamon, ginger, mace, nutmeg, oregano, black pepper, white pepper, rosemary, sage, savory, thyme, and turmeric.

Acknowledgment

The authors are indebted to the members of the Research Committee of the American Spice Trade Association and the companies they represent for generously supplying all the samples of spices that have been used in this investigation.

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NUTRIENT CONTENT OF CANE AND BEET SUGAR PRODUCTS*

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The use of sugar in the diet dates back to a very early period in man's history, and its importance in the diet of modern man is indicated by the per capita consumption of nearly a hundred pounds of sugar per year for the last 40 or more years (Figure 1). In the continental United States

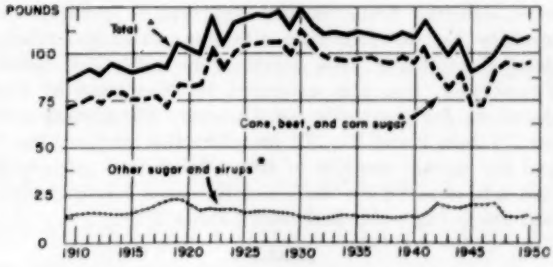

Figure 1. Sugar and sirups: annual per capita distribution, 1909-50. This table was reproduced from Clark, Friend and Burk, U.S.D.A. Miscellaneous Publication No. 616, January 1947, p. 28, and augmented by data from Miscellaneous Publication No. 691, August 1949, its supplement dated September 1950, and The National Food Situation, Bureau of Agricultural Economics, No. 57, July-September 1951.

*** "Other sugar and sirups" includes cane sirup, sorgo, edible molasses, maple sugar and sirup, refiners' sirup, honey and corn sirup.**

we produce cane sugar in 2 states and beet sugar in 16 states with California and Colorado competing closely for the leading position. Beet sugar provides about 20-25% of our total U. S. consumption *(8)*. As produced in modern mills, refined beet and cane sugar are identical.

The relative efficiencies of various agricultural products can be compared by tabulating the areas of land necessary for the production of one million calories in one year. The million calorie figure is derived from the fact that a full grown, moderately active man requires just about one million calories of food in a year. The importance of sugar as a supplier of calories is evidenced by the well known fact that the two commercial sugar producing plants, the beet and the cane, provide by far the largest food yield when this is expressed as calories per acre of land. This is reflected also in another way; namely, that sugar provides 1000 calories at the lowest cost compared with other common food stuffs. In addition to its caloric value, sugar plays a role in nutrition by contributing to the acceptance of other foods, particularly by children.

* This study was made possible through a grant in aid provided by the Sugar Research Foundation, New York, New York.

Refined sugar, on the other hand, does not provide vitamins, proteins, amino acids, lipids, and minerals; in fact, it has long had the reputation of being one of the purest organic substances manufactured on a large scale. Partially because of the "naked purity" of refined sugar and in part because of the extravagant claims and pseudoscientific statements and publications of food cultists and faddists, raw, crude, and "integral" sugars have been promoted as sources of vitamins, minerals, or some mystical health-giving substance. The lack of information in the scientific literature concerning the vitamin content of the various products produced during sugar manufacture is perhaps the best evidence for the lack of validity of many extravagant claims.

Because of this lack of facts concerning the amounts of important nutrients in sugar products, numerous representative samples of such products were collected from various geographic localities and assayed in this laboratory for the following vitamins: thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, biotin, folic acid, and inositol. Sugar Research Foundation, Inc. also arranged to have some of these samples analyzed elsewhere for inorganic constituents. These data are presented in this paper (Tables 1 and 2). To complete this picture, the Foundation also arranged for certain samples of spray-dried cane juice to be analyzed for important amino acids by the Food Research Laboratories. The data thus obtained are being published here (Table 3).

TABLE 1

Ash constituents of sugar products[1,2]

Constituent	Blackstrap molasses	Raw sugar	Brown soft sugar
Ash, total (% of original sample)	9.83	0.49	1.76
Element			
Potassium as K	23.37	18.54	27.18
Calcium as Ca	9.20	9.64	5.00
Magnesium as Mg	3.09	2.79	1.81
Silicon as Si	1.94	2.47	0.21
Phosphorus as P	0.47	0.57	0.19
In per cent of ash by spectrographic analysis			
Iron	0.40	0.50	0.35
Sodium	0.38	0.30	1.5[3]
Manganese	0.13	0.20	0.20
Rubidium	0.045	0.040	0.048
Aluminum	0.035	0.13	0.042
Copper	0.027	0.045	0.032
Lead	0.013	0.015	0.013
Barium	0.007	0.008	0.007
Strontium	0.005	0.006	0.005
Lithium	less than 0.001	0.005	0.003
Nickel	not found	not found	not found

Elements not found spectroscopically although definitely looked for and detectable in amounts indicated in parentheses were: zinc (0.05-0.01%); antimony (0.005%); arsenic (0.05%); bismuth (0.0005%); cobalt (0.005%); cadmium (0.005%); indium (0.005%); zirconium (0.01%).

[1] Analyses by a commercial laboratory for Sugar Research Foundation, Inc.

[2] Data already published may be found in references Nos. *3, 4, 7, 9, 11, 12, 13, 14, 15, 19, 23, 38, 39, 40, 41, 43, 45, 46, 49.*

[3] Extrapolated above highest standard. Figure is only approximate.

TABLE 2

Results of spectrographic analysis of sugar and molasses samples[1]

	Si	Mg	Mn	Fe	Al	Cu	Ni	Ti	Ag	Ca	Sr	Na	Rb	K
1. Hawaiian raw sugar 1945	m	M	m	m	m	m	tr?	–	tr	M	tr	m	M	m
2. Puerto Rico raw cane 1946	m	M	m	m	m	m	tr?	–	m–	M	tr	m–	m–	m
3. Peruvian raw sugar 1946	M	M	m	m	m	m	tr?	–	m–	M	tr	m	M	m
4. Cuban raw cane sugar 1946	m	M	m	m	m–	m	tr?	–	m–	M	tr?	m–	M–	m
5. Dominican Rep. raw cane sugar	m	M	m	m	m	m	tr?	–	m–	M	tr	m–	M–	m
6. Louisiana raw sugar 1946	M	M	m	m	m	m	tr?	–	m–	M	tr	m–	M–	m
7. Chinook, Mont., beet raw sugar	m	M	m	m	tr	m	tr?	–	m–	M	tr	m	M–	m
8. Toppenish, Wash., beet raw sugar	m	M	m	m	tr	m	tr?	–	M–	M	tr	m	M	m
9. West Jordan, Utah, beet raw sugar	m	M	m	m	m	m	tr?	–	m–	M	tr	M–	M	m
10. Louisiana raw sugar 1947 crop	m	M	m	m	tr	m	tr?	–	m–	M	tr?	m–	M–	m
11. Santo Domingo raw sugar 1947	m	M	m	m	m	m	tr?	–	m–	M	tr	m	M	m
12. Cuban raw sugar 1947	m	M	m	m	m	m	tr?	–	tr	M	tr	m	M	m
13. Puerto Rican raw sugar 1947	m	M	m	m	tr	m	tr?	–	m–	M	tr	m	M–	m
14. Peruvian raw sugar 1947	m	M	m	m	m	m	tr?	–	m–	M	tr	m	M	m
15. Chinook, Mont., beet molasses 1947	m	M	m	m	m	m	tr?	–	m–	M	tr	M–	M	m
16. Hi test molasses ⁋1655	m	M	m	m	m	m	tr?	–	m–	M	tr	m	M	m
17. Refinery molasses 1946	M	M	m	m	m	m	tr?	m	–	M	tr	m	M	m
18. Island black strap molasses 1946	M	M	m	m	m	m	tr?	–	–	M	tr	M–	M	m
19. Hi test molasses ⁋1748	m	M	m	m	m	m	tr?	–	–	M	tr	m	M	m
20. Blackstrap molasses Cuba Yale ⁋143	M	M	m	m	m	m	tr?	tr	tr	M	tr	m	M	m
21. Hi test molasses ⁋1694	M	M	m	m	m	m	tr?	–	–	M	tr	m	M	m
22. West Jordan beet molasses 1947	m	M	m	m	m–	m	tr?	–	m–	M	tr	M–	M	m
23. Hi test molasses ⁋1656	m	M	m	m	m	m	tr?	–	m–	M	tr	m	M	m
24. Blackstrap molasses, Dom. Rep. Yale ⁋45	M	M	m	m	m	m	tr?	–	m–	M	tr	m	M	m
25. Blackstrap molasses, Cuba Yale ⁋139	M	M	m	m	m	m	tr?	–	tr	M	tr	m	M–	m
26. Hi test molasses ⁋1673	m	M	m	m	tr	m	tr?	–	m–	M	tr	m	M–	m
27. Blackstrap molasses, Dom. Rep. Yale ⁋44	M	M	m	m	m	m	tr?	–	m–	M	tr	m	M	m
28. Hi test molasses ⁋1707	m	M	m	m	tr	m	tr?	–	tr	M	tr	m	M–	m
29. Blackstrap molasses, Puerto Rico Yale ⁋63	m	M	m	m	m	m	tr?	–	m–	M	tr	m	M	m
30. Blackstrap molasses, Puerto Rico Yale ⁋65	M	M	m	m	m	m	tr?	–	–	M	tr	m	M	m
31. Toppenish, Washington beet molasses 1947	m	M	m	m	m	m	tr?	–	m–	M	tr	M–	M	m

M—Probable major constituent of ash (Greater than 0.03% of sample)
m—Probable minor constituents of ash (Greater than 0.0003% of sample)
tr—Probable trace constituent of ash (Less than 0.0003% of sample)

[1] Analyses made by Dr. J. Rand McNally, Jr., Massachusetts Institute of Technology, Cambridge, Mass.

TABLE 3

Amino acids in spray dried cane juice[1]

Determination	Values found	
	γ per g	%
Total nitrogen[2]		0.117
Amino acids		
Leucine[3]	25	0.0025
Isoleucine[3]	10	0.0010
Valine[3]	18	0.0018
Lysine[4]	>40	0.0040
Tryptophane[5]	34	0.0034
Methionine[6]	9	0.0009
Arginine[6]	>20	0.0020
Threonine[6]	>15	0.0015
Histidine[6]	> 8	0.0008
Phenylalanine[6]	> 8	0.0008

[1] Processed at Instant Drying Corp., Poughkeepsie, N. Y., from juice extracted at Dobbs Ferry, N. Y. Analyses made by Food Research Laboratories, Long Island City, N. Y.
[2] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists (1945).
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[5] Greene and Black, *J. Biol. Chem.*, **155**, 1 (1944).
[6] Calculated on as received basis.

EXPERIMENTAL PROCEDURE

Materials investigated. Some 240 samples representative of the numerous sugar producing areas were used in the investigation and included the following products: *(a)* blackstrap molasses, *(b)* high test molasses, *(c)* refinery blackstrap molasses, *(d)* raw sugar, *(e)* turbinado sugar, *(f)* "integral" sugar, *(g)* Indian "Gur" sugar, *(h)* un-Steffenized beet molasses, *(i)* Steffenized beet molasses, *(j)* sugar beet press juice, *(k)* sugar beet diffusion juice, and *(l)* "low raw" sugar from sugar beets. Details of sugar manufacture from sugar cane and sugar beets, including flow sheets, are available *(35)*. All the steps in making both raw and refined sugar are carefully designed to have no effect whatever on the sugar itself, but merely to remove non-sugar materials.

For purpose of identifying the samples analyzed the following description is helpful. After the juice is expressed from the canes, it must be processed promptly to remove acids which would otherwise invert the sucrose. In addition to acids, the juice contains plant lipids, some amino acids, and minerals. Purification is generally effected by heating the juice to near boiling after which ordinary lime is added. Precipitates with organic acids, phosphates and sulfates are formed and as the precipitate settles, most of the wax globules and fiber particles are entrapped and all the non-sugar material is filtered off with relative ease.

The "clarified juice" is then evaporated in partial vacuum to the point where sugar crystals separate. Three or four separate "crops" are thus produced and separated from most of the adhering molasses by spinning in high speed centrifuges.

Although the *raw sugar* so prepared in modern mills is usually more than 97% pure, as ordinarily handled and shipped, it is not considered, under the pure Food and Drug regulations, as suitable for direct human consumption. Large numbers of microorganisms such as yeast, molds, and bacteria, as well as lint and cane fiber, may be present. Each crystal has a thin film of slightly sticky material clinging to the surface and the color ranges from pale yellow to various shades of brown. *Blackstrap molasses* is the last material remaining from the concentrated sugar cane juice after all the sugar commercially possible to recover, has been removed. All of the reboiling which results finally in blackstrap molasses produces much caramelization of sugars and gives a bitter product of rather poor palatability. Data on the composition of blackstrap molasses are given in Table 4. The composition of the ash varies considerably depending on soil and processing conditions (Table 5). *High test molasses* consists of whole cane juice inverted and boiled down to a solids content of about 85%. The term "*integral*"

TABLE 4

Analyses of blackstrap and high test molasses

Type of molasses and source	Sucrose	Invert sugar	Total sugar	Brix solids	Thiamine	Riboflavin	Pyridoxine	Niacin	Pantothenic acid	Biotin	Folic acid	Inositol
	%	%	%	%	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
Blackstrap Cuba (1947)	35.6	14.1	49.8	84.3	0.55	3.57	1.37	16.7	30.2	1.23	0.021	1.62
" " "	37.3	14.2	51.5	85.6	0.83	2.34	1.39	17.5	39.3	1.04	0.029	1.47
" " "	37.2	14.1	51.2	85.5	0.86	2.23	1.42	13.9	44.2	1.08	0.031	1.52
" " "	35.3	21.3	56.7	90.0	1.30	2.71	1.57	14.2	31.5	0.87	0.048	1.68
" " "	39.6	20.4	60.0	90.7	0.87	1.36	0.95	17.4	36.4	0.72	0.032	1.41
" " "	36.7	20.8	57.5	89.5	1.15	1.44	1.58	17.6	22.9	0.96	0.032	1.37
" " "	33.5	21.6	55.1	85.0	0.81	1.66	1.42	14.6	44.8	0.97	0.036	1.61
" " "	35.8	22.1	57.9	87.5	0.75	1.12	1.15	22.5	43.1	0.99	0.014	1.42
" " "	41.4	16.0	57.4	90.6	0.95	1.27	1.22	24.1	43.8	1.27	0.033	1.19
" " "	37.6	21.2	58.8	89.7	0.60	1.21	1.17	26.6	63.9	1.60	0.021	1.41
" " "	37.2	22.8	59.9	88.8	0.53	0.83	1.22	27.2	42.2	1.70	0.012	1.62
" " "	35.8	23.7	59.5	87.0	1.10	2.67	1.25	28.5	47.5	1.44	0.051	1.52
" " "	37.2	17.8	54.9	88.3	0.63	2.31	1.02	25.1	42.2	1.21	0.013	1.47
" " "	35.5	21.7	57.2	89.6	1.30	2.22	1.24	23.8	40.7	1.42	0.044	1.49
" " "	33.9	23.7	57.6	88.6	1.10	2.13	1.37	20.3	45.7	1.38	0.048	1.62
" " "	37.8	20.3	58.1	88.8	0.97	3.19	1.49	21.2	64.1	1.45	0.077	1.39
" " "	36.7	26.1	62.8	87.7	1.30	2.51	1.37	17.2	58.8	1.27	0.018	1.21
" " "	34.0	27.2	61.2	88.2	0.80	1.85	1.83	16.9	52.4	1.26	0.041	1.37
" " "	36.4	21.3	57.7	87.4	0.91	3.20	1.45	29.6	46.1	1.54	0.069	1.62
" " "	37.8	15.8	53.6	88.0	2.30	1.56	2.20	22.2	47.2	1.11	0.019	1.57
" " "	42.3	19.5	61.8	85.9	0.82	2.56	1.10	16.0	55.0	1.23	0.029	1.49
" " "	35.2	19.5	54.7	82.1	0.78	3.42	2.41	28.2	35.2	1.02	0.032	1.42
" " "	40.5	16.4	56.9	86.9	1.60	2.02	1.60	24.9	51.0	1.52	0.032	1.62
" " "	33.8	26.7	60.5	87.0	1.70	2.06	1.90	21.0	55.9	1.47	0.023	1.42
" " "	37.2	16.7	53.9	87.5	1.20	2.15	2.10	19.5	43.0	2.11	0.047	1.37
" " "	40.2	13.2	53.3	87.0	2.70	2.71	1.91	22.3	32.8	2.10	0.023	1.64
" " "	37.8	17.2	55.0	89.5	2.00	2.61	2.30	23.2	40.0	1.51	0.054	1.50
" " "	37.5	22.9	60.3	88.0	1.80	2.34	1.30	17.4	40.7	1.03	0.021	1.39
" " "	39.0	14.3	53.3	86.0	0.80	1.64	1.80	22.0	45.0	1.43	0.064	1.62

TABLE 4 (continued)

Type of molasses and source	Sucrose	Invert sugar	Total sugar	Brix solids	Thiamine	Riboflavin	Pyridoxine	Niacin	Pantothenic acid	Biotin	Folic acid	Inositol
	%	%	%	%	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
Blackstrap Cuba (1947)	31.5	24.8	56.4	85.5	0.85	2.57	2.30	24.0	52.5	1.32	0.020	1.28
" " "	38.7	17.7	56.3	86.0	1.80	2.75	1.60	21.4	42.1	1.50	0.097	1.32
" " "	37.2	21.2	58.3	84.7	1.1	1.67	1.70	22.2	53.1	1.61	0.026	1.41
" " "	36.1	24.3	60.4	88.0	1.3	2.29	1.50	28.4	43.8	1.48	0.021	1.38
" " "	33.2	23.3	56.6	85.9	1.01	2.38	2.31	26.1	45.8	2.01	0.050	1.31
" " "	35.5	18.9	54.4	88.6	1.30	1.72	2.08	21.6	39.5	2.02	0.050	1.42
" " "	42.4	15.5	57.9	88.8	2.10	1.56	1.58	26.0	22.4	1.48	0.047	1.37
" " "	35.3	19.6	54.9	85.5	1.01	2.08	1.95	26.5	42.5	1.63	0.028	1.29
" " "	38.2	20.1	58.3	86.2	0.90	1.32	2.15	19.9	36.0	1.50	0.057	1.44
" " "	35.9	24.3	60.2	86.6	0.98	2.42	2.06	27.1	34.1	1.21	0.042	1.36
" " "	38.8	23.1	61.9	87.7	0.84	2.30	1.98	25.0	37.6	1.20	0.037	1.47
" " "	33.4	26.8	60.2	87.4	1.30	1.62	1.72	23.0	54.7	1.51	0.065	1.32
" " "	41.1	20.4	61.5	87.1	0.96	2.22	1.20	24.5	38.0	1.44	0.017	1.36
" " "	37.8	15.2	53.0	86.4	0.96	1.27	1.48	21.1	25.9	1.72	0.024	1.71
" " "	34.0	26.2	60.2	86.0	1.11	1.41	1.75	15.8	46.5	1.79	0.035	1.42
" " "	38.0	23.5	61.4	86.4	0.69	1.23	1.45	18.3	31.4	1.82	0.023	1.27
" " "	37.3	12.7	50.0	87.8	1.13	1.95	1.55	23.7	31.5	1.83	0.076	1.31
" " "	45.2	15.0	60.1	84.6	0.95	1.89	1.83	18.8	21.9	1.17	0.037	1.62
" " "	38.7	13.3	52.0	86.2	0.96	1.60	1.93	25.8	23.9	1.80	0.075	1.41
" " "	36.4	20.2	56.6	87.5	1.19	1.62	2.55	18.6	31.4	1.23	0.043	1.40
" " "	34.9	22.5	57.3	85.6	0.79	1.86	1.33	22.9	33.6	1.50	0.086	1.28
" " "	39.4	18.0	57.4	90.2	1.91	2.06	1.83	21.5	35.7	1.75	0.088	1.31
" " "	38.8	18.3	57.1	88.0	0.76	1.69	2.13	19.6	27.4	1.38	0.051	1.47
" " "	42.9	20.3	63.2	84.4	0.97	1.34	1.58	16.5	33.5	1.12	0.020	1.57
" " "	42.0	18.8	60.8	87.1	0.99	1.35	1.50	17.6	22.5	1.11	0.057	1.42
" " "	40.9	17.4	58.4	88.6	2.32	2.18	2.45	20.8	40.0	1.30	0.041	1.36
" " "	38.9	18.7	57.7	86.2	2.12	2.28	1.73	19.9	45.9	1.02	0.068	1.27
" " "	33.5	21.6	55.1	89.0	0.65	1.53	2.06	15.0	40.9	2.37	0.033	1.37
" " "	39.7	18.5	58.2	87.5	0.88	1.52	1.90	17.1	39.2	2.00	0.036	1.24
" " "	37.2	22.9	60.0	87.3	1.19	2.26	2.04	17.8	41.9	2.16	0.037	1.36

TABLE 4 (continued)

Type of molasses and source	Sucrose	Invert sugar	Total sugar	Brix solids	Thiamine	Riboflavin	Pyridoxine	Niacin	Pantothenic acid	Biotin	Folic acid	Inositol
	%	%	%	%	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
Blackstrap Cuba (1947)	36.0	22.4	58.4	86.3	0.85	1.59	1.84	17.6	41.6	1.84	0.035	1.41
" " "	39.0	17.3	56.3	87.4	0.92	1.86	2.37	18.6	36.0	1.45	0.038	1.50
" " "	34.9	19.1	54.0	87.9	0.70	1.91	2.39	23.6	42.4	1.60	0.036	1.32
" " "	40.4	18.6	59.0	87.2	0.81	1.49	1.93	18.5	23.5	1.21	0.035	1.41
" " "	39.4	18.7	58.1	88.9	0.94	2.24	1.70	20.0	24.0	0.80	0.056	1.34
" " "	34.5	24.2	58.7	86.8	2.89	2.26	1.80	18.0	25.4	0.91	0.050	1.26
" " "	39.1	11.7	50.8	86.5	0.81	2.23	1.71	19.3	45.7	1.48	0.080	1.42
" " "	36.7	25.0	61.7	85.1	1.14	2.15	1.80	15.8	33.7	1.17	0.033	1.31
" " "	38.4	18.4	56.8	87.9	0.80	2.09	1.60	17.9	45.1	1.37	0.041	1.41
" " "	41.2	15.5	56.7	87.9	0.80	2.07	1.30	18.6	34.4	1.22	0.036	1.47
" " "	35.9	22.7	58.6	87.5	0.80	1.82	1.81	20.0	31.2	1.61	0.021	1.26
" " "	34.6	22.4	56.9	86.9	0.91	1.33	2.04	18.5	35.9	1.34	0.069	1.29
" " "	38.2	16.6	54.8	88.3	0.94	1.20	1.70	17.2	27.5	0.63	0.028	1.35
" " "	35.0	21.9	56.9	85.9	0.90	2.07	2.50	21.0	16.1	0.69	0.038	1.42
" " "	41.1	16.4	57.5	81.5	0.82	1.60	1.70	18.9	18.8	0.60	0.027	1.50
" " "	34.9	22.0	56.9	82.1	0.75	1.89	1.60	15.4	20.5	0.70	0.039	1.46
" " "	33.6	19.6	53.2	84.6	0.84	1.71	1.71	13.6	19.6	0.70	0.034	1.37
Peru (1947)	34.0	18.7	52.6	85.2	0.51	1.01	1.21	18.6	33.6	0.73	0.033	1.41
Dom. Rep. (1947)	31.4	24.3	55.7	83.7	0.66	2.49	1.59	15.3	27.4	1.42	0.028	1.36
" " "	31.4	24.3	55.7	83.6	0.85	1.09	1.55	19.7	32.6	1.31	0.011	1.47
" " "	34.9	23.4	58.3	89.3	1.11	1.70	1.80	25.4	29.7	1.90	0.041	1.50
" " "	29.4	27.3	56.7	85.9	1.10	0.98	1.69	20.3	18.5	1.30	0.019	1.46
" " "	31.7	25.9	57.6	86.3	0.89	0.73	1.56	18.0	30.7	1.24	0.022	1.34
" " "	30.5	30.2	60.7	87.0	1.40	1.48	1.56	22.0	37.1	1.16	0.061	1.27
" " "	32.0	29.7	61.7	86.6	0.65	1.55	1.50	17.3	43.4	1.44	0.018	1.31
" " "	32.2	24.4	56.6	86.9	2.10	1.84	2.25	21.3	44.0	1.92	0.050	1.43
" " "	32.5	20.4	52.9	86.8	0.99	1.50	1.34	23.5	29.6	1.64	0.013	1.37
" " "	33.5	21.5	55.4	89.6	1.13	1.20	1.47	15.1	39.4	1.19	0.032	1.29
" " "	32.5	23.4	55.9	83.6	0.74	1.58	1.50	25.6	27.4	0.73	0.029	1.41

TABLE 4 (concluded)

Type of molasses and source	Sucrose	Invert sugar	Total sugar	Brix solids	Thiamine	Riboflavin	Pyridoxine	Niacin	Pantothenic acid	Biotin	Folic acid	Inositol
	%	%	%	%	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
Puerto Rico (1947)	33.1	23.8	56.9	86.0	0.85	2.71	1.72	22.7	44.3	1.37	0.024	1.38
" " "	32.9	22.6	55.5	87.1	0.80	2.90	1.74	16.7	52.8	1.13	0.014	1.49
" " "	32.9	18.8	51.7	85.7	0.96	2.51	2.00	25.8	47.0	1.58	0.067	1.27
" " "	35.9	22.9	58.8	91.3	1.80	2.65	1.80	21.3	47.4	1.39	0.055	1.41
" " "	35.9	20.9	56.8	89.3	0.83	2.17	1.50	19.0	37.8	1.76	0.035	1.12
" " "	35.9	25.4	61.3	87.4	1.70	2.30	1.61	26.9	55.7	1.72	0.050	1.36
" " "	33.2	23.4	56.6	86.2	0.84	2.00	1.68	24.8	47.4	1.63	0.072	1.60
" " "	35.2	20.6	55.7	87.0	0.87	2.32	1.50	25.3	44.4	1.61	0.047	1.36
" " "	33.8	19.1	52.9	86.2	1.05	2.37	1.55	22.1	31.0	1.56	0.054	1.42
" " "	33.5	21.6	55.1	89.0	1.02	2.14	2.15	28.1	43.2	1.84	0.063	1.27
" " "	34.1	18.9	53.0	86.5	0.64	2.23	2.07	25.1	56.7	1.55	0.021	1.34
Blackstrap												
Haiti (1947)	31.4	21.9	56.3	86.3	0.79	1.61	1.47	18.0	51.7	1.92	0.018	1.28
Hawaii (1947)	33.7	16.1	49.8	76.2	2.41	3.64	2.06	27.4	42.8	1.46	0.071	1.64
Refinery Blackstrap												
Charleston, Mass. (1947)	37.3	21.5	68.8	79.6	0.08	0.15	0.37	0.93	1.7	0.07	0.002	
Hawaii (1947)	34.9	16.3	51.2	79.1	0.24	1.06	1.02	14.1	12.6	0.72	0.014	
High Test Molasses	27.6	44.2	71.8	83.8	0.32	1.02	1.53	3.1	3.9	0.38	0.012	
" " "	16.4	59.7	76.1	85.1	0.34	0.75	1.20	2.6	4.9	0.35	0.010	
" " "	24.1	52.5	76.6	85.3	0.45	0.32	1.21	4.6	3.3	0.29	0.007	
" " "	26.0	50.9	76.9	85.3	0.36	0.65	0.93	4.6	4.3	0.29	0.010	
" " "	27.3	50.3	77.6	86.1	0.33	0.53	1.22	3.8	5.4	0.38	0.011	
" " "	16.8	59.7	76.6	85.2	0.31	0.75	1.14	3.0	4.0	0.40	0.009	
" " "	24.3	48.9	73.1	84.0	0.35	0.78	1.18	4.2	4.4	0.41	0.009	
" " "	25.6	51.3	76.9	85.1	0.38	0.42	1.06	4.9	2.9	0.27	0.010	
" " "	27.8	44.1	71.9	83.9	0.35	0.25	1.57	2.7	3.7	0.27	0.011	
" " "	20.0	54.8	74.9	84.0	0.35	0.33	1.24	2.7	4.3	0.30	0.020	

TABLE 5

Analyses of raw sugars

Source or identity of samples	Sucrose	Ash	Organic non-sugars	Dry weight	Thiamine	Riboflavin	Pyridoxine	Niacin	Pantothenic acid	Biotin	Folic acid
	%	%	%	%	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
Composite Samples (Raw) (1946)[1]											
Cuba	97.2	0.53	0.89	99.4	>0.1	0.46	0.33	5.35	1.80	0.061	0.014
Puerto Rico	97.5	0.50	0.61	99.4	>0.1	0.48	0.44	3.15	1.82	0.062	0.012
Santo Domingo	97.3	0.40	0.87	99.4	>0.1	0.47	0.22	4.35	1.65	0.054	0.018
British W. Indies	96.8	0.55	0.73	99.2	0.18	0.50	0.29	4.20	1.95	0.052	0.024
Peru	96.8	0.86	0.84	99.3	0.16	0.60	0.84	3.05	1.50	0.064	0.022
Composite Samples (Raw) (1947)[2]											
Cuba	97.1	0.52	0.79	99.3	>0.1	0.46	1.08	3.90	1.31	0.092	0.023
Puerto Rico	96.4	0.81	0.68	99.6	>0.1	0.33	0.97	4.68	2.11	0.071	0.018
Santo Domingo	97.2	0.62	0.74	99.4	0.13	0.30	0.35	4.29	1.58	0.070	0.019
British W. Indies	97.4	0.54	0.83	99.2	>0.1	0.35	0.85	4.12	2.14	0.070	0.029
Composite Sample (Raw) (1948)[3]											
Brazil	97.3	0.44	0.66	99.3	>0.1	0.20	0.92	3.6	3.96	0.080	0.018
Louisiana	97.3	0.63	0.44	99.4	>0.1	0.27	0.75	4.3	5.57	0.090	0.012
Peru	97.4	0.87	0.32	99.5	>0.1	0.26	0.82	3.8	2.97	0.110	0.019
Miscellaneous Raw											
Florida	.....	.....	.....	98.2	0.18	0.29	0.24	4.70	1.14	0.028	0.014
Hawaiian	.....	.....	.....	99.1	0.16	0.25	0.48	3.05	2.10	0.051	0.012
Louisiana	.....	.....	.....	98.9	0.27	0.21	0.28	4.15	2.75	0.035	0.016
Hakalan	.....	.....	.....	93.0	<0.1	0.41	0.69	3.42	3.10	0.141	0.012
Gay-Rob	.....	.....	.....	98.0	<0.1	0.27	0.34	2.86	3.40	0.094	0.017
Hilo Sugar	.....	.....	.....	94.0	<0.1	0.37	0.67	3.91	1.50	0.058	0.012
Hutchenson	.....	.....	.....	98.0	<0.1	0.35	0.47	4.02	3.12	0.111	0.011
Kilauea	.....	.....	.....	95.0	<0.1	0.29	0.45	2.84	2.50	0.065	0.019
Olokele	.....	.....	.....	98.0	<0.1	0.16	0.50	2.98	1.60	0.060	0.014
Paaukau	.....	.....	.....	98.0	<0.1	0.15	0.37	4.32	1.70	0.070	0.018
National	.....	.....	.....	91.0	<0.1	0.47	0.80	2.73	1.55	0.090	0.027

[1] Cuban sample—a composite from 38 factories; Puerto Rican sample—a compose. Raw sugar samples analyzed were submitted by a number of factories in each country. Cuba—38 factories; Puerto Rico—14 factories; Santo Domingo—3 factories; British West Indies—10 producers.

[2] Samples composited from producer as follows: Cuba—57; Puerto Rico—19; Santo Domingo—7; British West Indies—14.

[3] Samples composited from producers as follows: Brazil—"various," the individual factories not specified; Louisiana—10; Peru—7.

sugar has been applied to various experimental products prepared by drying cane juice. *Turbinado sugar* is raw sugar that has been washed a little more than the raw sugar generally prepared for shipment to the refineries, and consists of crystals of nearly pure sugar with a thin coating of molasses and probably a certain amount of molasses entrapped within.

After the raw sugar is shipped to the refinery, it is first washed to remove the film of adhering syrup, dissolved in water and treated with charcoal. The charcoal is removed by filtration, leaving a sparkling, colorless thin syrup. From such syrups several crops of pure white sugar crystals (refined sugar) can be separated by evaporation under vacuum. Finally, the crystallization process yields yellow or brown crystals coated with a film of syrup containing salts, coloring matter, and flavored substances. These yellow and brown sticky crystals are generally given little or no washing and are marketed as the various grades of "soft" sugars: "yellow," "light yellow," "golden yellow," "light brown," "old-fashioned brown," etc. They are clean, wholesome products and are fully refined in the sense that they have been treated in the refinery to remove undesirable impurities. The boiled down residue after crystallization of the "soft" sugars is *refinery blackstrap*. *Indian "Gur" sugar* is a product comparable to, but much cruder than, raw sugar as described above.

The preparation of sugar from sugar beets depends first on extraction of the sugar by counter current dialysis, and the juice resulting is termed *diffusion juice*. *Press juice* is obtained for laboratory purposes by simply pressing the juice from the sugar beet. The *"low raw" sugar* from sugar beets is prepared from diffusion juice by clarification procedures similar to the preparation of raw sugar from sugar cane. This product is of course then refined as is raw cane sugar. *Un-Steffenized* molasses is the boiled-down residue after several crystallizations of sucrose from the beet juice. The Steffens process, which results in *Steffenized molasses*, consists of adding quick lime to the original beet molasses to throw out a precipitate of calcium saccharates. The precipitate is filtered and the sugar is recovered by treatment with CO_2 or else the calcium saccharate is used to replace lime in the original clarification treatment of diffusion juice.

SAMPLING PLAN

The problem of obtaining a "representative sample" of a natural product always poses considerable difficulty, owing to variations that may exist in the vitamin content within any species, and even variety of a species. Although the scope of literature on the vitamin content of sugar products is very limited, data that are available *(1, 6, 10, 16, 17, 18, 20, 22, 28, 29, 31, 32, 34, 36, 37, 44, 47, 48)* indicate that the vitamin content of cane juice varies both with geographic location and varieties of sugar cane. Although the variation in vitamin content is often large percentage wise, in terms of actual quantity the differences are of a very low order of magnitude. In addition, similar vitamin-destructive processes are applied generally in the production of raw sugar so that much of the variation of vitamin content that exists in the original juice is nullified. In any event, every effort was made to obtain representative samples of the products analyzed. This is perhaps best illustrated by the compositing of the raw sugar samples analyzed which represented the production of a large number of factories of each country or geographic location (see footnote, Table 5). To illustrate this point further, sample 7 of Steffenized molasses from Billings, Montana (Table 6) was an average sample from an area represented by the Yellowstone River valley from Columbus to Hysham. Consideration of year-to-year variation is evidenced by the analyses of composite raw sugar samples from the production years, 1946, 1947, and 1948 (Table 4). In all cases, at least, a sample consisted of one pint or one pound of the material tested. After the samples were collected and transported to the Yale Nutrition Laboratory, they were stored in a cold room maintained at 9-10°C. Perishable samples of fresh cane juice were packed in dry ice and shipped air express. These samples were stored in the frozen state at –10°C.

METHODS OF ANALYSES

The analytical data on total solids, sucrose, invert sugar, total sugar, ash, and organic non-sugars were conducted in the New York Sugar Trade Laboratory, Inc. under the supervision of Dr. F. W. Zerban, according to standard accepted methods described by the rules and regulations for the Analysis of Molasses and Syrups.

TABLE 6

Analyses of beet sugar products

Source and identity of product	Sugar	Dry weight	Thiamine	Riboflavin	Pyridoxine	Niacin	Pantothenic acid	Biotin	Folic acid
	%	%	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
Un-Steffenized molasses (1946-47)									
1 Alma	50.8	83.6	<0.1	1.20	4.6	41.7	7.20	0.035	0.150
2 Crosswell	49.5	82.6	<0.1	1.29	5.8	49.5	9.10	0.053	0.184
3 Sebewaing	50.6	84.0	<0.1	1.35	5.9	47.8	16.7	0.069	0.153
4 Bagard	56.6	81.7	<0.1	0.76	3.2	42.3	13.7	0.014	0.160
5 Scottsbluff	58.8	83.1	<0.1	0.88	3.9	45.6	7.52	0.021	0.146
6 Eaton	54.0	91.6	<0.1	0.70	4.1	47.8	13.5	0.028	0.120
8 Lowell	52.8	84.1	<0.1	0.98	4.0	57.7	10.2	0.019	0.172
11 East Grand Forks	N.A.	84.0	<0.1	1.04	4.5	31.4	16.1	0.024	0.290
12 Chaska	N.A.	82.0	<0.1	0.83	5.2	53.0	15.2	0.029	0.168
14 Missoula	N.A.	81.0	<0.1	1.53	3.8	54.5	11.6	0.029	0.200
17 Blackfoot	50.6	80.0	<0.1	0.62	4.9	50.9	3.14	0.040	0.184
18 Chinook	49.1	79.8	<0.1	0.73	3.9	51.1	4.22	0.027	0.270
19 Belle Fourche	49.9	79.6	<0.1	0.64	3.6	56.3	6.87	0.021	0.179
20 Gunnison	49.6	78.8	<0.1	0.90	3.2	42.7	5.61	0.029	0.176
21 West Jordan	51.1	82.2	<0.1	0.90	3.3	36.2	17.6	0.029	0.362
22 Toppenish	48.2	81.2	<0.1	1.14	5.4	47.9	13.5	0.038	0.178
Steffenized molasses (1946-47)									
7 Billings	57.4	78.8	<0.1	0.48	4.0	41.1	3.30	0.014	0.064
10 Mason City	N.A.	80.0	<0.1	0.22	4.3	33.4	5.12	0.023	0.084
13 Rocky Ford	N.A.	80.0	<0.1	0.46	2.7	30.1	4.84	0.012	0.028
15 Oxnard	N.A.	84.0	<0.1	0.55	3.4	25.6	7.61	0.029	0.048
16 Grand Island	N.A.	81.0	<0.1	0.42	3.3	30.1	11.8	0.035	0.033
23 Garland	50.1	80.2	<0.1	0.69	2.7	39.9	7.41	0.027	0.067
9 Fort Collins	N.A.	79.1	<0.1	0.72	3.0	31.0	10.6	0.018	0.024
Steffens waste conc.	N.A.[1]	N.A.	Neg.[2]	Neg.	1.94	53.4	5.4	0.047	0.034

[1] N.A.—Not available.
[2] Neg —Negligible.

TABLE 6 (concluded)

Source and identity of product	Sugar	Dry weight	Thiamine	Riboflavin	Pyridoxine	Niacin	Pantothenic acid	Biotin	Folic acid
	%	%	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
(Fractions obtained in production of beet sugar)									
West Jordan, Utah									
115 Press juice	14.2	16.6	0.91	0.52	0.66	1.59	3.34	0.006	0.014
116 Diffusion juice	12.4	14.6	0.93	0.43	0.54	0.01	2.56	0.004	0.019
117 Molasses	50.5	82.0	>0.1	0.90	3.72	43.7	7.95	0.100	0.112
118 Low raw sugar	91.2	99.2	>0.1	0.51	0.71	9.86	2.82	0.042	0.073
Chinook, Montana									
119 Press juice	17.3	19.2	0.51	0.35	0.52	1.96	2.05	0.002	0.011
120 Diffusion juice	11.8	13.6	0.46	0.42	0.55	1.55	1.65	0.003	0.015
121 Molasses	51.7	83.6	>0.1	0.64	1.60	49.9	2.96	Neg.	0.143
122 Low raw sugar	91.4	98.2	>0.1	0.26	1.30	11.1	2.60	Neg.	0.018
Toppendish, Washington									
124 Press juice	15.9	18.1	0.42	0.51	0.36	1.72	1.16	0.002	0.030
125 Diffusion juice	15.9	15.8	0.38	0.35	0.51	1.67	1.43	0.002	0.025
126 Molasses	49.8	82.2	>0.1	1.14	1.80	56.4	7.94	Neg.	0.196
127 Low raw sugar	89.2	97.0	>0.1	0.46	1.40	13.3	1.15	Neg.	0.077
West Jordan, Utah									
134 Press juice	14.7	17.0	0.47	0.36	0.50	2.36	1.58	0.002	0.013
135 Diffusion juice	12.1	13.3	0.39	0.28	0.48	2.27	1.14	0.001	0.011
136 Molasses	49.8	81.8	>0.1	0.86	3.96	39.1	8.62	Neg.	0.284
137 Low raw sugar	93.6	99.0	>0.1	0.21	0.65	9.1	1.77	Neg.	0.031
Toppendish, Washington									
161 Press juice	13.5	15.8	0.64	0.29	0.51	1.74	2.33	0.002	0.050
162 Diffusion juice	12.7	14.4	0.49	0.18	0.42	1.60	1.20	0.001	0.030
163 Molasses	48.0	81.6	>0.1	1.19	1.34	47.20	8.20	Neg.	0.312
164 Low raw sugar	90.4	97.2	>0.1	0.39	0.96	15.8	1.71	Neg.	0.059
Chinook, Montana									
165 Press juice	17.2	18.2	0.64	0.41	0.57	2.76	2.86	0.002	0.059
166 Diffusion juice	12.5	13.1	0.52	0.42	0.36	2.68	2.38	0.001	0.050
167 Molasses	51.9	81.7	>0.1	1.05	1.80	47.5	8.30	Neg.	0.309
168 Low raw sugar	92.1	97.4	>0.1	0.28	0.86	14.5	2.56	Neg.	0.049

[1] N.A.—Not available.
[2] Neg.—Negligible.

TABLE 7

Analyses of brown and soft sugars

Source or identity and laboratory No.	Sucrose	Invert sugar	Dry weight	Ash	Organic non-sugar	Thiamine	Riboflavin	Pyridoxine	Niacin	Pantothenic acid	Biotin	Folic acid
	%	%	%	%	%	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
204 Old fash. brown	87.1	7.1	98.5	1.86	2.42	0.14	0.64	0.41	3.04	2.14	0.054	0.005
202 Old fash. brown	87.5	7.3	98.5	1.13	2.57	0.15	0.40	0.58	2.41	1.42	0.031	0.008
213 Old fash. dk. brown	91.9	4.0	98.8	1.41	1.60	0.21	0.16	0.46	1.89	1.02	0.091	0.005
201 Old fash. dk. brown	91.2	4.3	98.9	1.59	1.85	>0.1	0.44	0.65	2.20	2.21	0.074	0.007
216 Dark brown	89.2	5.0	98.7	1.70	2.76	>0.1	0.16	0.61	1.68	2.51	0.164	0.014
206 Dark brown	89.5	5.1	98.7	1.81	2.24	0.23	0.84	1.36	3.64	2.70	0.074	0.010
215 Medium brown	91.6	4.1	99.0	1.25	2.03	>0.1	0.20	0.27	0.71	2.10	0.061	0.013
212 Medium brown	92.7	3.6	99.0	1.10	1.66	0.37	0.27	0.37	1.70	2.40	0.119	0.004
214 Light brown	92.2	3.9	99.0	1.09	1.92	>0.1	0.21	0.29	0.63	1.82	0.050	0.008
211 Light brown	91.9	4.3	98.7	1.11	1.37	1.0	0.16	0.32	1.00	3.50	0.064	0.010
205 Light brown	92.0	3.9	98.9	1.00	2.03	0.11	0.40	0.56	2.50	1.32	0.037	0.011
221 Light brown	93.2	3.2	98.9	1.16	1.36	70.1	0.18	0.30	1.84	2.50	0.062	0.007
209 Golden brown	92.5	3.6	98.9	1.35	1.48	0.13	0.52	0.64	1.82	2.02	0.048	0.006
223 Golden brown	95.3	2.3	99.2	0.61	1.04	>0.1	0.13	0.39	0.72	1.01	0.035	0.006
210 #3 yellow	89.3	4.9	98.7	2.02	2.51	0.56	0.65	0.82	2.80	1.64	0.062	0.004
219 Brown soft	88.7	4.7	98.4	2.55	2.48	>0.1	0.16	0.68	1.96	4.41	0.102	0.007
218 Yellow soft	89.6	4.1	98.3	2.59	2.09	>0.1	0.13	0.23	1.21	3.60	0.064	0.016
217 Canary soft	94.3	2.8	99.1	0.84	1.13	0.12	0.24	1.24	1.41	1.20	0.044	0.005
225 Soft, gr. #13	87.5	5.5	98.4	1.94	3.48	>0.1	0.17	1.08	1.92	2.50	0.046	0.004
224 Soft, gr. #10	94.0	2.9	99.2	0.94	1.41	>0.1	0.13	0.41	1.04	1.40	0.036	0.005

TABLE 7 (concluded)

Source or identity and laboratory No.	Sucrose	Invert sugar	Dry weight	Ash	Organic non-sugar	Thiamine	Riboflavin	Pyridoxine	Niacin	Pantothenic acid	Biotin	Folic acid
	%	%	%	%	%	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
228 Golden yel. (KT)	92.4	2.9	97.4	0.91	1.19	0.12	0.13	0.42	0.84	1.10	0.046	0.012
227 Best brown	89.7	3.1	96.8	1.60	2.38	>0.1	0.25	0.45	1.36	3.80	0.090	0.004
226 Golden yellow	91.0	3.3	97.4	1.39	1.74	>0.1	0.15	0.22	0.40	2.00	0.042	0.064
229 Best brown (KT)	90.7	3.5	97.5	1.23	2.05	>0.1	0.16	0.87	0.80	1.60	0.058	0.012
230 Kleenraw	94.6	1.9	99.3	1.30	1.48	>0.1	0.29	0.62	0.72	1.30	0.025	0.003
232 Yellow D	86.8	6.0	98.2	3.18	2.26	>0.1	0.17	0.63	1.44	3.20	0.046	0.006
231 Golden C	89.0	4.7	98.3	2.28	2.31	>0.1	0.17	0.86	1.36	3.21	0.120	0.002
233 Extra C	91.8	3.4	98.7	2.01	1.54	>0.1	0.14	0.54	1.32	2.20	0.044	0.003
207 Golden yellow	93.3	3.4	99.1	1.09	1.37	0.13	0.56	0.60	1.60	1.71	0.052	0.008
208 C3 yellow	89.5	5.6	98.8	1.68	1.99	0.23	0.44	0.54	2.20	2.31	0.039	0.004
203 Brilliant yellow	88.3	6.0	98.4	2.02	2.11	>0.1	0.16	0.57	1.80	2.00	0.033	0.015
222 Golden yellow	94.6	2.5	99.1	0.99	1.08	>0.1	0.16	0.33	2.32	1.30	0.090	0.014
220 Light yellow	97.3	1.2	99.6	0.50	0.52	>0.1	0.17	0.38	1.01	1.20	0.064	0.016
25 Yellow D	92.0	....	93.0	.....	.....	>0.1	0.24	0.53	1.13	2.31	0.118	0.021
26 Golden C	91.2	....	92.0	.....	.....	>0.1	0.22	0.26	1.14	2.64	0.094	0.014
27 Extra C	94.1	....	96.0	.....	.....	>0.1	0.23	0.56	1.19	2.32	0.085	0.016
173 Light brown	.....	....	95.8	.....	.....	<0.1	0.68	0.82	2.6	3.42	0.010	0.005
174 Dark brown	.....	....	96.2	.....	.....	<0.1	0.57	1.22	3.3	4.41	0.014	0.004
175 Yellow	.....	....	94.8	.....	.....	<0.1	0.56	0.50	2.8	3.90	0.019	0.005
176 Old fash. dark	.....	....	96.2	.....	.....	<0.1	0.60	0.96	2.6	4.38	0.020	0.003
177 Medium brown	.....	....	94.8	.....	.....	<0.1	0.68	0.58	1.7	3.74	0.060	0.007

For the vitamin analyses, 2 to 5 gm. quantities of sample were weighed out and assayed as outlined below. Microbiological methods were used for riboflavin, pyridoxine, niacin, pantothenic acid, folic acid and inositol. A thorough review of the methodology of microbiological assay procedures has been presented by E. C. Barton-Wright *(5)*.

Thiamine. The sample was prepared by heating for 30 minutes at 100°C. with 75 ml. of 0.1 NHCl, after which the solution was cooled to 50°C., and incubated 2 hours with Takadiastase. Thiamine was then determined by the thiochrome method as outlined by Methods of Vitamin Assay *(26)*.

Riboflavin and niacin. Samples were prepared by autoclaving with 10 volumes of 0.1 NH_2SO_4 for 30 minutes at 121°C. After neutralization to pH 7, the samples were diluted to appropriate volume and assayed for riboflavin and niacin using the test organisms *Lactobacillus casei* and *Lactobacillus arabinosus* respectively. The medium employed was that of Krehl and Fruton *(25)*. Pure riboflavin and niacin were used as the standards.

Biotin and inositol determination. Samples were prepared by autoclaving with 10 volumes of 2 NH_2SO_4 for 2 hours at 121°C. Although somewhat more drastic treatment of the sample has been suggested for the liberation of biotin and inositol *(42)*, longer periods of autoclaving with more concentrated acid did not increase significantly the values for biotin and inositol, and it was concluded that the treatment employed provided maximum liberation of the respective vitamins.

After autoclaving, the samples were cooled, neutralized to pH 5, diluted to such a volume as to fall within the range of the standard curves and assayed as follows:

Inositol was determined with the yeast *Saccharomyces cerevisiae* as the test organism. The medium used was that employed by Atkins *et al. (2)*, for the yeast *Saccharomyces carlsbergensis*, modified by the addition of pyridoxine and removal of inositol. Growth was measured by turbidity, as determined with the Evelyn Photoelectric Colorimeter, using a filter with maximum transmission at 620 mμ.

L. arabinosus was the test organism used for the determination of biotin, using the biotin free medium of Krehl and Fruton *(25)*. The graded production of lactic acid, in response to the biotin present in the sample, was compared to the pure vitamin and used as the end-point for the assay.

Folic acid. For the determination of folic acid the samples were adjusted to pH 7.5 with 1 N NaOH, 25 ml. of distilled water added, and the solution autoclaved for 1 hour at 121°C. The volume of the solution was adjusted so as to fall within the assay range of the test organism *Lactobacillus casei*, using pure folic acid as a standard. The medium employed was the folic acid free medium of Krehl and Fruton *(25)*. The treatment of samples with hog kidney conjugase, as suggested by Olson *et al. (30)*, did not reveal a significantly greater content of folic acid in the sugar products tested and was, therefore, abandoned, since it is considerably more cumbersome. Whether or not the procedure employed is completely adequate for liberation of bound folic acid in sugar products is debatable; however, since amounts of this vitamin found are of such a small order of magnitude, extensive study on this aspect of the problem did not seem justified.

Pyridoxine. Pyridoxine samples were prepared by adding 24 ml. of 0.1 N H_2SO_4, after which the solution was autoclaved at 121°C. for one hour. After cooling, the pH of the solution was adjusted to 5 and the sample diluted to such a volume that three levels of the diluted sample fell within the assay range of the test organism *S. carlsbergensis*, when pure pyridoxine was used as the standard. The medium of Atkins *et al. (2)* was used, and growth of the yeast as turbidity was measured as in the inositol assay. It should be noted that this organism measures all three forms of the vitamin, (i.e. pyridoxal, pyridoxamine, and pyridoxine).

Although longer periods of autoclaving have been recommended for the liberation of vitamin B_6 from certain types of material, *(33)*, the sample treatment outlined above gave maximum pyridoxine values for the sugar products tested.

Pantothenic acid. The presence of bound forms of pantothenic acid in biological materials poses the problem of liberating pantothenic acid so that it can be determined microbiologically. The early analyses for pantothenic acid employed the method of enzymatic liberation of Ives and Strong *(21)*. Subsequent work *(27)* indicated that treatment of the biological material with an intestinal phosphatase and an enzyme extractable from acetone powder of chicken liver resulted in an increased liberation

TABLE 8

Miscellaneous sugar products

Source and identity	Sugar	Dry weight	Thiamine	Riboflavin	Pyridoxine	Niacin	Calcium pantothenate	Biotin	Folic acid
	%	%	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.	γ/g.
Cuba									
Dehydrated cane juice	70.5	80.8	0.48	0.62	1.00	4.81	7.90	0.135	0.091
Louisiana									
Cane juice	13.8	21.4	0.09	0.12	0.29	1.02	3.70	0.034	0.028
Spray dried cane juice	75.2	87.0	0.18	0.39	1.04	3.97	13.9	0.114	0.084
Cane juice—lab. sample[1]	16.2	19.8	0.09	0.18	0.71	1.34	2.40	0.044	0.038
Cane juice—lab. sample[2]	15.4	23.6	0.13	0.17	0.55	1.87	1.53	0.022	0.005
Cane juice—dehydrated[2]	77.8	99.1	0.52	0.56	1.96	7.62	7.76	0.075	0.029
Turbinado sugar	99.4	99.9	Neg.	0.14	0.11	1.30	0.80	0.008	0.012
Integral sugar	70.5	80.1	0.14	0.46	1.18	9.01	4.20	0.014	0.014
Indian gur sugar #1	75.6	93.6	>0.1	0.99	1.50	1.56	3.85	0.025	0.130
Indian gur sugar #2	77.5	92.5	>0.1	0.77	1.17	1.86	4.69	0.020	0.076

Neg.—Negligible.

[1] Sugar cane, preserved by a waxed coating, was cut into small pieces and ground in the Waring blender to a fine pulp from which the juice was expressed by squeezing through several layers of cheese cloth.

[2] Processed at Instant Drying Corporation, Poughkeepsie, N. Y., from cane juice extracted at Dobbs Ferry, N. Y.

of pantothenic acid. Application of this method did indeed result in slightly higher pantothenic acid values for samples which contained a relatively high level of pantothenic acid, and for the most part this liberation procedure was used. The high "blank" values, however, which resulted from the chicken liver enzyme preparation did not permit satisfactory application of this method to samples containing minimal amounts of pantothenic acid.

After liberation of pantothenic acid, this vitamin was determined microbiologically by the use of the test organism *L. arabinosus* and the medium of Krehl and Fruton *(25)*. Pure calcium pantothenate was used as the standard.

RESULTS AND DISCUSSION

The analytical data obtained are compiled in Tables 1-8. After examining these results it must be concluded that, at least from a broad point of view, raw sugars, soft sugars, turbinado sugars, "integral" sugar, and even cane juice itself, dried carefully so as to preserve as much as possible the vitamins therein, do contain vitamins, but in such small quantities as to be completely impractical as a nutritional source of these important substances. The heating and lime treatment applied to cane or beet juice in the process of sugar preparation contribute largely to the destruction of vitamins such as thiamine, riboflavin, and calcium pantothenate. This is well borne out by the findings that the thiamine content of raw sugar is reduced to the vanishing point. The following illustrates how ridiculous it is to consider these crude sugar products as a reasonable source of vitamins. If one takes as a basis of reference the 1953 recommended daily dietary allowances of the Food and Nutrition Board of the National Research Council for thiamine, riboflavin, and niacin—namely 1.5, 1.6, and 15 mg., respectively—the daily consumption of one pound of raw sugar would provide practically no thiamine, and about 10% and 12% of the recommended allowances of riboflavin and niacin, respectively. Again, using the data obtained from the assays, the daily ingestion of one pound of dehydrated cane juice itself, spray dried so as to minimize vitamin loss, would provide only about 12%, 13%, and 23%, respectively, of the thiamine, riboflavin, and niacin recommended allowances. Brown sugars, turbinado sugars, and other miscellaneous products would provide, of course, much smaller amounts of these vitamins. Of all the sugar products examined, blackstrap molasses, high test molasses, and un-Steffenized molasses offer the most interesting data. Blackstrap, and particularly molasses from sugar beets, contain appreciable amounts of niacin, but even here if one should be so misguided as to consume one pound of blackstrap molasses per day the reward would be about 64% of the recommended niacin allowance and 34% and 54% of the thiamine and riboflavin allowances, respectively. While molasses has important nutritional attributes for the animal husbandryman in the feeding of cattle for reasons other than its vitamin content, its role in human nutrition as a possible source of vitamins is indeed insignificant.

Since too little is known regarding the role of pyridoxine, pantothenic acid, biotin, folic acid and inositol in human nutrition, no one can say in what quantities these substances are needed. It is evident, however, judging from the amounts found in crude sugar products, that most of these vitamins are present in very insignificant quantities. It is interesting to note, however, that blackstrap molasses contains an appreciable amount

of inositol, making it in fact one of the richest sources of this compound. Since inositol is also found in liberal quantities in citrus juices *(24)* and many other natural products, a deficiency of this substance does not loom as very imminent on the nutritional horizon.

Differences in vitamin content of samples obtained from different geographic locations are not greater than the differences among samples within any one location, and since the amounts of vitamins present are very small, no significance can be attached to the differences obtained.

A look at the ash composition of molasses (Table 5) reveals that this product contains appreciable amounts of calcium, phosphorus, magnesium, and a fair amount of iron. The very large amounts of potassium usually present in blackstrap molasses undoubtedly limit the amount of molasses that could be ingested without disturbing mineral balances and causing undue laxation.

Results of the spectrographic analysis (Tables 1 and 2) of various sugar and molasses samples presented in Table 5 reveal magnesium, calcium, and, interestingly, rubidium as probable major constituents of the ash (greater than 0.03% of the sample). Calcium is also listed as a major constituent of the ash and its spectrum includes lines in the red end. It is possible that these may have caused some confusion with rubidium lines owing to the compression of the prismatic spectrum in the red. Further work is required to resolve this point.

The remote possibility that cane juice might contain amino acids in appreciable amounts is ruled out by the results shown in Table 3. It is obvious that they are present in trace amounts at best.

It is difficult to over-emphasize the role that nutrition has played in improving the general health and vital statistics of the population, and it is even more difficult to single out a more essential task for society than to assure good nutrition for all its members. It must be emphasized that nutrition as a science and a real contributor to the welfare of man must be based on sound scientific facts arrived at by reasonable and careful experimentation. The introduction of mysticism, cultism, superstition, and the emotional belief that some special system of eating can produce supermen results only in the exploitation of those who most need sound nutritional guidance.

SUMMARY

Approximately 240 samples of products resulting from the processing of cane or beet juice have been analyzed for their content of thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, biotin, folic acid, and inositol. It must be concluded that, although these products do contain vitamins, they are present in such small quantities as to be completely impractical as a significant nutritional source of these important substances.

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INFLUENCE OF MATURITY AND STORAGE TREATMENTS UPON THE ASCORBIC ACID CONTENT OF THE SEEDS OF SOUTHERN PEAS[a]

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The nutritional value of the Southern pea, *Vigna sinensis* L., depends to a large extent upon its maturity when harvested and its subsequent treatment. Its popularity is highest in the South where it is grown extensively; however, its potentiality as a source of food has not been exploited fully.

The Southern pea contains large quantities of certain essential vitamins as well as other dietary components which are necessary for a balanced diet *(2)*. Richardson *et al. (5)* reported an average of 53.3 mg. of ascorbic acid per 100 g. of peas on a dry weight basis and 21.7 mg. per 100 g. on a fresh weight basis for four varieties of green-shell Southern peas. Reid *(4)* reported 20.8 mg. ascorbic acid per 100 g. of ripening cowpea seeds, but found only a trace, if any, in the dry seeds of cowpeas.

EXPERIMENTAL

There are three general types of Southern peas; the blackeye, cream, and crowder. In this study the California Blackeye No. 5 variety was selected to represent the blackeye type, the cream type was represented by the Two-Crop Conch variety and the crowder type by the Alabama Crowder variety.

This experiment was divided into two phases, one dealing with changes in the ascorbic acid content of the seeds of Southern peas during their maturation and the other with the effects of storage conditions upon this vitamin. In order for the data to be analyzed statistically the phase of the experiment dealing with the maturity of Southern peas was set up in a randomized block design. The portion of the experiment dealing with the effect of storage upon the peas was designed as a $3 \times 3 \times 4$ factorial. It consisted of 3 varieties of Southern peas, 3 storage temperatures and 4 storage periods *(6)*. The storage temperatures were 40°, 70° and 100°F. Samples were taken at 0, 24, 48 and 72 hours. The relative humidity in the storage rooms ranged from approximately 85 to 90%. The data were analyzed by the analysis of variance techniques from which linear and curvilinear responses to maturity were tested *(6)*.

To facilitate harvesting the samples at 6 different stages of maturity, approximately 300 blooms in each variety were tagged and their date of flowering recorded. Pods which developed from the tagged flowers were utilized as indices of maturity for harvesting all samples. Peas representing the first stage of maturity were harvested as early in their development as they could be separated from the pericarp. This was approximately 10 days after flowering. At this stage, the contour of the seeds was barely visible on the sides of the pods. The hulls were very green in color at this point in their development and remained green until the fourth stage of maturity approximately 16 days after flowering. After peas representing the first stage of maturity were harvested, subsequent stages were harvested at two-day intervals. Southern peas utilized in the storage study were selected at a stage of maturity that closely resembled peas in maturity stage 4 of the maturity study. At this stage of development the seeds appeared to have reached their maximum size and the pods were ready to break from a green to a yellow-green color.

Ascorbic acid was determined by the indophenol method described by Bessey *(1)*, as modified by Menaker and Guerrant *(3)*.

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RESULTS AND DISCUSSION

Maturity. When Southern peas are utilized at an early stage in their development, they are a very good source of ascorbic acid. However, as the seeds approach a stage of field dryness there is little retention of this vitamin (Table 1). Although there were differences in the ascorbic acid

TABLE 1

Effect of maturity upon the ascorbic acid content of three varieties of Southern peas expressed as milligrams per 100 grams of seeds.

State of maturity		Variety					
		California No. 5		Conch		Alabama Crowder	
		Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
1		57.8	343.6	54.2	262.0	51.1	291.8
2		52.1	238.8	43.3	166.5	44.2	187.0
3		43.8	170.2	36.5	111.1	38.9	131.9
4		38.1	125.8	24.0	63.3	28.7	77.1
5		25.9	75.1	15.4	35.5	17.2	41.4
6		15.7	37.6	7.6	15.2	6.6	14.1
L.S.D.	.05	2.3	11.5	1.1	7.7	1.9	12.1
	.01	3.2	15.9	1.5	11.0	2.8	17.1
Coef. of variability		4.0%	4.7%	2.0%	3.9%	3.4%	5.3%

content among the 3 varieties studied in this experiment, the response of this vitamin to maturity changes followed the same general pattern in all varieties. During a period of approximately 10 days following the formation of the seeds in the pod of Southern peas, the ascorbic acid decreased 89.0% in the California No. 5 variety, on a dry weight basis. In the Conch variety there was a decrease of 94.2% and for the Alabama Crowder variety a decrease of 95.2%. Since this decrease with maturity on a dry weight basis followed a curvilinear rather than a linear relationship (Figure 1), the rate of decrease was greater in the first stages of maturity than in the latter. Because of this fact, the average per cent decrease per day was not apportioned evenly among all stages.

In comparing the results of this study with that of Richardson *et al. (5)*, their mean value of 53.3 mg. of ascorbic acid per 100 g. of seeds on a dry weight basis was approximately the same as the amount found in this study for peas which had just begun to lose their green color. Practically the same comparison could be made on a fresh weight basis. This would indicate that a slightly higher ascorbic acid content was found in this study since Richardson *et al.* reported their findings on mature-green shelled peas corresponding to maturity stage 4 of this study.

Storage. From the results shown in Table 2, it is evident that there was a continuous decrease in the ascorbic acid on both fresh and dry weight basis as a result of increased storage time. This was found to be the case for all 3 varieties studied. This decrease in the ascorbic acid with an increase in time followed a slightly curvilinear pattern rather than linear (Figure 2). On a percentage basis, this represented an average decrease in ascorbic acid of 34.0% during a period of 72 hours with a mean temperature of 70°F. When this retention was apportioned among the 3 varieties, the Alabama Crowder variety had the highest decrease with

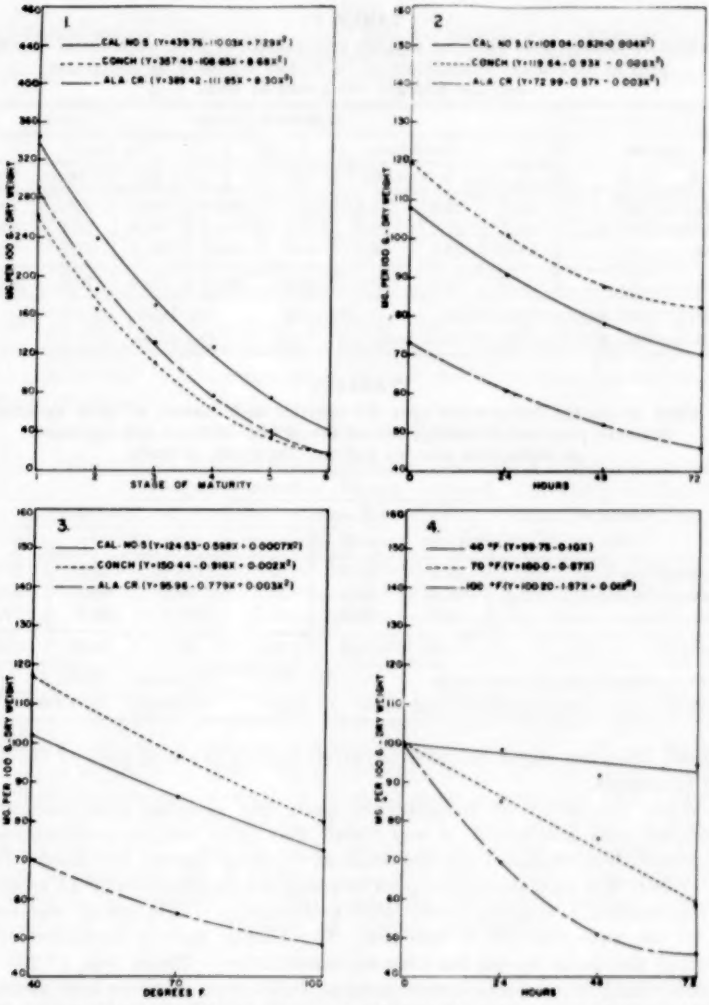

Figure 1. The relationship of maturity to the ascorbic acid content of three varieties of Southern peas.

Figure 2. The response of the ascorbic acid content of three varieties of Southern peas to storage time.

Figure 3. The response of the ascorbic acid content of three varieties of Southern peas to different storage temperatures.

Figure 4. The interaction of storage temperature and time upon the ascorbic acid content of Southern peas.

37.3% and the Conch variety the lowest with 31.0%. The decrease in the California No. 5 variety was approximately 35.1%. This response of the ascorbic acid was not proportionate throughout the storage period, but

TABLE 2

The effect of storage time upon the ascorbic acid content of three varieties of Southern peas at an average temperature of 70°F. and expressed as milligrams ascorbic acid per 100 grams of seeds.

Variety	Hours in storage							
	Fresh basis				Dry basis			
	0	24	48	72	0	24	48	72
California No. 5	35.0	28.9	25.2	23.0	108.1	90.6	78.2	70.1
Alabama Crowder	26.7	22.2	19.1	17.0	73.1	60.6	52.1	45.8
Conch	35.6	29.8	26.1	24.8	119.5	101.1	87.6	82.4
Mean	32.4	27.0	23.5	21.6	100.2	84.1	72.6	66.1

L.S.D. for time mean on fresh basis .01 = 0.6 Dry basis .01 = 2.0

L.S.D. for time within variety on fresh basis .01 = 1.0 Dry basis .01 = 3.5

TABLE 3

The effect of storage temperature upon the ascorbic acid content of three varieties of Southern peas held in storage for an average of 36 hours and expressed as milligrams ascorbic acid per 100 grams of seeds.

Variety	Storage temperature					
	Fresh basis			Dry basis		
	40°F.	70°F.	100°F.	40°F.	70°F.	100°F.
California No. 5	32.9	28.2	23.1	101.8	86.3	72.2
Alabama Crowder	25.4	20.8	17.5	69.6	56.1	48.0
Conch	34.7	28.8	23.7	117.1	96.4	79.4
Mean	31.0	25.9	21.4	96.1	79.6	66.5

L.S.D. for temp. mean on fresh basis .01 = 0.5 Dry basis .01 = 1.8

L.S.D. for temp. within variety on fresh basis .01 = 0.8 Dry basis .01 = 2.9

instead, the most rapid decrease occurred during the first part of the storage treatment.

When the effect of temperature upon the ascorbic acid content of Southern peas was tested, it was found that there was an acceleration in the loss of this vitamin from the seeds as the temperature increased (Table 3). When this response to temperature was shown graphically (Figure 3), it represented a slightly curvilinear relationship. This trend was found to be the same for all 3 varieties. The Conch variety appeared to be affected the most by an increase in temperature. There was 17.2% less ascorbic acid in peas which were held at 70°F. than in those held at 40°F., and 30.8% less when held at 100°F. than when held at 40°F. The rate of decline in the ascorbic acid on a dry weight basis was almost twice as great in peas held at 100°F. as for those held at 70°F. during the first 48 hours.

When the interaction of time and temperature was considered (Table 4), it was found that relatively little ascorbic acid was lost over a period of 72 hours when Southern peas were held at 40°F. However, when they were held at a temperature of 70°F., there was a 15.4% decrease during the first 24 hours and only about 58% remained at the end of 72 hours on a dry weight basis. When peas were held at 100°F., there was a decrease of approximately 31% of the ascorbic acid content during the first 24 hours and only about 46% remained at the end of 72 hours. The

TABLE 4

The effect of storage temperature and time upon the ascorbic acid content of Southern peas expressed as milligrams per 100 grams of seeds.

Storage temperature	Hours in storage							
	Fresh basis				Dry basis			
	0	24	48	72	0	24	48	72
40°F.	32.4	31.2	29.7	30.6	100.2	98.3	91.7	94.4
70°F.	32.4	27.5	24.5	19.3	100.2	84.8	75.5	58.0
100°F.	32.4	22.1	16.2	14.9	100.2	69.2	50.7	45.8
L.S.D. .05		0.7			L.S.D. .05		2.7	
L.S.D. .01		1.0			L.S.D. .01		3.5	

decline in the ascorbic acid of Southern peas held in storage at 40 and 70°F. appeared to follow a linear relationship. However, when the peas were held at 100°F., the response of the ascorbic acid formed a curvilinear pattern (Figure 4).

SUMMARY AND CONCLUSIONS

The influence of maturity and storage treatments upon the ascorbic acid content of the Southern pea, *Vigna sinensis*, was studied. Samples were harvested at six different stages of maturity. Those representing the first stage of maturity were harvested as soon as the seeds could be separated from the pericarp. Peas representing the subsequent stages of maturity were harvested at two-day intervals until the sixth or final stage was harvested. The storage treatments consisted of three varieties of Southern peas held in storage for periods of 0, 24, 48, and 72 hours at temperatures of 40, 70, and 100°F.

Results showed that Southern peas are relatively high in ascorbic acid if they are harvested at an early stage in their development and utilized immediately or held in storage at approximately 40°F. If they are allowed to develop to the point where the green color begins to disappear from the pod, little benefit can be derived from them as a source of this vitamin.

When the pods were held at 40°F. for 72 hours, there was relatively little decrease in the ascorbic acid content of the seeds. However, as the storage temperature was increased to 70 and 100°F., there was a decrease of 42 and 54%, respectively. An increase in the time of storage had the same general effect as increasing the storage temperature.

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EFFECT OF MOISTURE AND HIGH TEMPERATURE ON CELL WALLS IN PLANT TISSUES

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Two divergent viewpoints exist regarding the physical effect of cooking on the structure of cell walls in plant tissues. On the one hand, the claim is made that the softening of tissues is due primarily to the easy separation of the cells and secondarily to the loss of rigidity in the individual cell walls. Since the intercellular cement is generally conceded to be composed in most part of pectic substances *(1, 2, 7)*, any agent or process which breaks down those substances can obviously bring about cell separation. Likewise, the demonstrable presence of pectic materials as a "filling" material in the primary wall *(12, 23)* is one basis for assuming that the wall itself will become softer as pectic materials are degraded.

Work with bacterial and fungal rots *(8)* has shown that the softening in the host tissues is associated with the secretion of pectolytic enzymes by the rot organisms. Kertesz *(8)* has reviewed investigations showing that heat can also cause the breakdown of pectic substances. Quantitative measurements of this thermal degradation (in terms of tensile strength of tissues) have been made by Personius and Sharp *(13)*. Their microscopic observations *(14)* and those of Day *(4)*, Butler *et al. (3)*, Sweetman *(21)* and Sterling and Bettelheim *(20)* on cooked potato tissues have demonstrated the separation of intact cells during the cooking process.[a] Reeve and Leinbach *(17)* also find this to be the case in apple.

On the other hand, various observers have stated that rupture of the cell wall generally attends the cooking of plant tissue. Hutchison *(5)* and more recently Sherman *(18)* have shown similar figures illustrating the rupture of cell walls in potato as a consequence of cooking. In other vegetables, Simpson and Halliday *(19)* likewise depict the breaking of walls of parenchymatous cells during cooking. Since the primary wall of these cells in fleshy tissues is composed principally of a cellulose skeleton and pectic matrix *(1, 12, 23)*, these observations would indicate a possible degradation of cellulose in the cooking process.[b]

In order to obtain conclusive evidence on the effects of cooking, several food products were subjected to steaming procedures and examined microscopically. The results of such examination constitute the subject of this communication.

EXPERIMENTAL PROCEDURE

The materials investigated in this study were carrot, potato, and apple. The carrot variety was Imperator (California); the potato, Russet Burbank (Idaho) and White Rose (California); and the apple, Delicious (Washington). These materials were sliced into disks about 1 cm. thick and then steamed for 20, 40 and 60 minutes in an

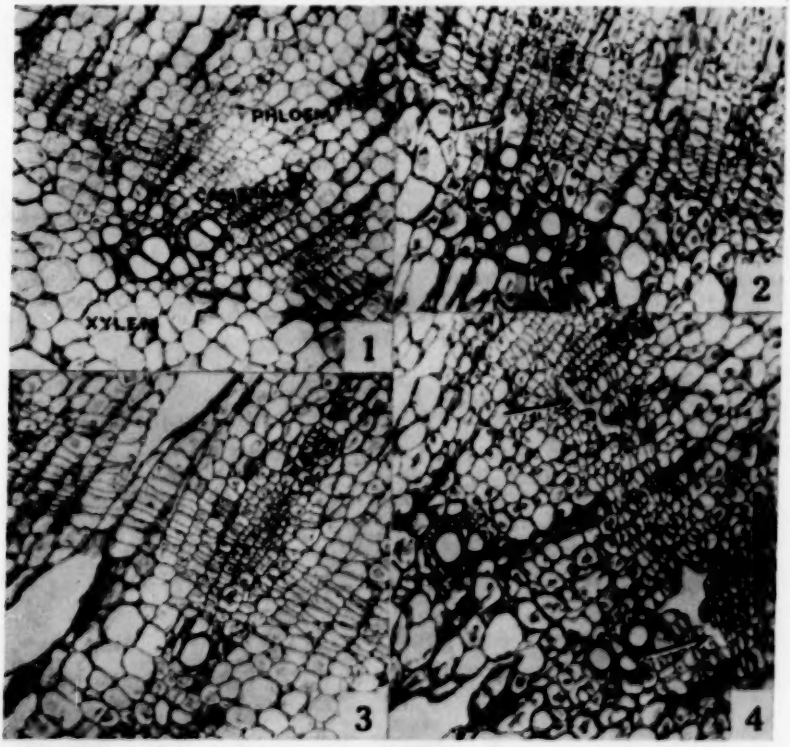
[a] See also the figures given by Longrée *(9)* and Reeve *(16)*.

[b] Although polysaccharides other than pectins and cellulose occur in plant cell walls, their nature is not clear at the present time.

autoclave at atmospheric pressure. In addition, some potato segments were boiled for periods up to one hour, and whole potatoes were baked for 1 hour at 375°F. Sections 1 × 1 × 0.5 cm. were carefully cut from raw and cooked pieces and placed in Randolph's solution *(6)* of a mixture of chromic acid, formalin, and acetic acid for preservation for two days. After being washed out in water, they were gradually dehydrated with successively increasing concentrations of ethanol, cleared with successively increasing concentrations of xylol, infiltrated gradually with paraffin, and finally embedded in the latter. Microtome sections were cut at 10 μ, and these were stained with iron haematoxylin *(15)*.

RESULTS

Carrot. Figures 1-4 give the successive stages in carrot steaming, from the raw piece (Figure 1) through the 20-minute (Figure 2) and the 40-minute steaming (Figure 3) to the 60-minute steaming (Figure 4). All sections are from the cambial region of the carrot, *i.e.*, including the meristematic (cell-producing) layer lying between the



In Figures 1-4, xylem is towards bottom of page in each. Figures represent various times of steaming in cross section of carrot root. All × 120.

Figure 1. Raw carrot showing typical tissue architecture.

Figure 2. Steamed 20 minutes. Arrow indicates cleavage between radial rows of cells in cambium.

Figure 3. Steamed 40 minutes. Separation of radial rows apparent in xylem and phloem.

Figure 4. Steamed 60 minutes. Arrows point to regions of tangential separation of cambial cells. Note radial separation nearby in xylem.

inner core of xylem and the outer ring of phloem. Hence all the bulk tissues of the carrot are represented here.

In Figure 1, the cells are intact, and occasional small intercellular spaces are observable in the older xylem and phloem tissues farther away from the cambial ring. The group of thick-walled xylem elements near the center of the figure are vessel elements, whose walls are heavily lignified.

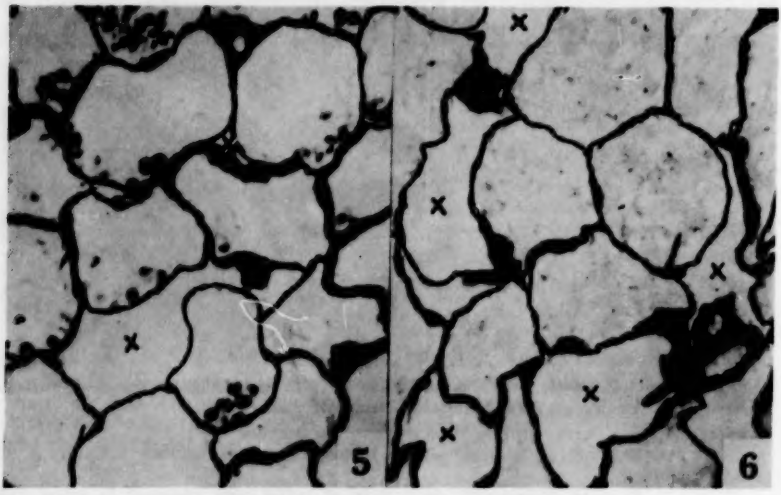
Figure 2 shows the marked coagulation of the protoplasts of the cells after 20 minutes of steaming. In addition, it may be seen (at the arrow) that some separation of the radial rows of cells is taking place in the cambial region. The walls of these cells are more delicate than in other tissues, and presumably the pectic intercellular materials are not as well-developed as elsewhere. Note that there is otherwise no indication of cell wall rupture. Noticeable wrinkling of the parenchymatous walls presumably is due to plasmolytic action during preparation of the material for cutting. It probably indicates weakening of the walls as a result of steaming.

After 40 minutes of steaming (Figure 3), it is apparent that radial separation of cells occurs in xylem and phloem tissues as well. Again, it is obvious that cell separation is the principal change taking place in the architecture of the tissue. No broken cells are noticeable.

When the tissues are steamed for 60 minutes (Figure 4), the carrot segments become quite soft. Microscopically, it is apparent that the extent of cell separation has increased considerably. Not only are there extensive areas of radial cell separation throughout the segments, but in the more delicately-walled cambial region, a tangential separation of cells is also taking place (at arrows). Again, it will be seen that there is no breakage of cells walls such as is figured for carrots after 45 minutes of steaming by Simpson and Halliday *(19)*.

Apple. Since the rapid onset of mushiness in cooked apples is well-known, it seems reasonable to expect that cell wall rupture may occur during the cooking of its tissues. Figure 5 portrays tissue of a raw apple. Starch grains are apparent in the cell interiors and air spaces among the cells (at X). The wrinkled shape of the cells again indicates a plasmolytic effect in preparing the material for sectioning.

Following a 60-minute steaming period, as shown in Figure 6, the starch grains have been gelatinized. The gelatinous mass fills the cells, giving them a grayish cast



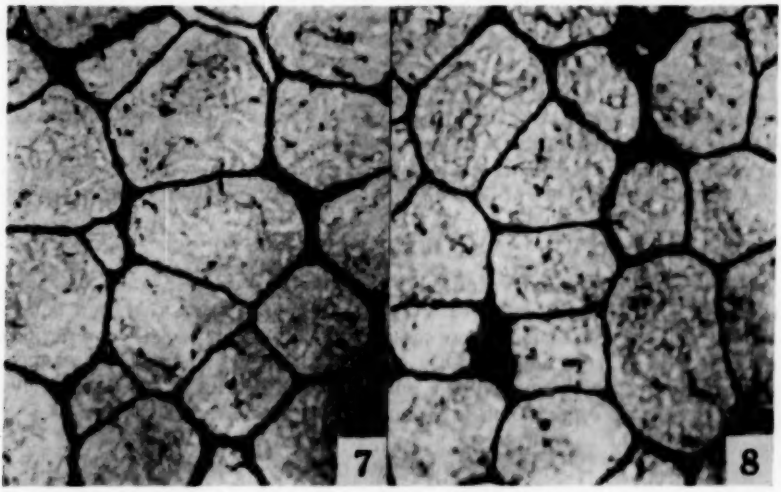
Cross sections of parenchymatous flesh of apple fruit. × 125.

Figure 5. Raw apple, with starch grains in cells. "X" denotes intercellular space.
Figure 6. Apple steamed 60 minutes. "X" as in Figure 5.

as counterposed to the white intercellular spaces. The weak cell walls are more extensively folded and wrinkled. However, no sign of cell wall breakage is apparent in the apple. Only the extent of intercellular spaces is increased due to cellular separation.

Potato. In view of the many studies of potato tissue behavior during cooking, the effect of a long cooking time on potato segments was particularly studied. Figure 7 shows a portion of the interior of a Russet Burbank potato after boiling for 1 hour. Note that although the cells are beginning to separate, their walls are still intact. In Figure 8, there is illustrated a portion of the tissue of boiled White Rose potato. Here cell separation is much less evident, occasional cell interstices showing the presence of small air spaces. Again, no cell wall rupture can be seen.

With minor variations, the steamed and the baked potatoes show exactly the same results as do the boiled, even after 60 minutes of treatment.



Cross sections of parenchymatous flesh of potato tuber, boiled 60 minutes. × 125.
Figure 7. Russet Burbank with incipient cell separation.
Figure 8. White Rose with little evidence of cell separation.

DISCUSSION

The evidence of this study indicates that cell walls are not broken down by heat and moisture in the cooking process. Hence, it is presumable that the cellulose skeletal network is likewise unimpaired. Simpson and Halliday *(19)* decide for a change in the cellulose (to form hydrolytic products) on steaming because of the resultant greater thinness, and even breakage, of the cell walls and their deeper staining with standard microchemical reagents. However, it is quite reasonable to suppose that some of these changes may readily be explained by the dissolution of the incrusting pectic materials, with little effect on the cellulose. More pertinently, Tauss *(22)* cooked cellulosic materials for 3 hours at ordinary pressures, at 5 atmospheres, 10 atmospheres, and 20 atmospheres of pressure. Even at 5 atmospheres (152°C.), little if any cellulose dissolution occurred. Only at 10 atmospheres was there any noticeable breakdown of cellulose, at which point 13.48% of the cellulose as such was decomposed. Finally, at 20

atmospheres, "hydrocellulose" was formed, and the whole mass became gelatinous.

Butler *et al. (3)* have pointed out that many later interpretations of cell wall breakage in cooking of potatoes are based on the original figures of Maercker *(10)*. In this instance, Maercker showed cells of potato cooked for one hour. The cells were separated in his Figure 138, but some showed broken walls. However, his legend indicates that the potatoes (after steaming) had been squeezed through rollers. His own text *(10)* does not mention wall rupture, as a consequence of cooking, but only cell separation, due to the dissolution of intercellular pectic substances. A later edition *(11)* also stresses that in cooking, the cell walls of potato are preserved and only rarely do cells burst. At 3 atmospheres pressure, some breakdown of cell walls may occur, according to his figures.

Perhaps the breakage in cell walls, shown by Simpson and Halliday *(19)*, may have been due to handling of the material in preparation of microscopic sections, to nicks in the edges of the microtome knife, etc. (see Reeve, *16)*. However, Reeve *(16)* has found that cell ruptures can occur in potato tissues as a result of swelling of gelatinized starch. Nevertheless, he does not find evidence of cell wall breakdown as a result of cooking *per se*.

SUMMARY

The effect of moisture and high temperature on cell walls of plant tissues was studied microscopically with apple fruit, carrot root, and potato tuber. These were steamed, and occasionally otherwise cooked, for periods up to one hour. In no case were cell walls ruptured. In each instance, tissue architecture was modified only by the separation of intact cells. Explanations of contrary observations were discussed.

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EFFECT OF DEGREE OF ENZYME INACTIVATION AND STORAGE TEMPERATURE ON QUALITY RETENTION IN FROZEN PEAS

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The necessity of blanching (scalding) vegetables to be preserved by freezing has been recognized since the report by Joslyn and Cruess *(12)* in 1929. The deterioration in flavor and color and the off-odors which develop in raw or under-blanched vegetables during freezing storage have been ascribed to the action of enzymes. In many instances, the enzyme sytem or systems responsible for the deteriorative changes have not been identified. Nevertheless, it has become common practice to use certain enzyme tests as indices of adequacy of heating in vegetables to be preserved by freezing. Some question still remains as to which enzyme best serves this purpose.

Diehl and Berry *(6)* in 1933 stated that catalase activity served as an index of adequacy of blanching for peas and indicated that Alderman peas blanched for a time sufficient to destroy catalase activity retained satisfactory quality when stored at –5°F. (–20.6°C.). Later, Arighi, Joslyn, and Marsh *(1)* observed that catalase appeared to be quantitatively inactivated at a much more rapid rate than the enzymes concerned in the production of off-flavors in peas. In view of these findings, Joslyn *(11)* suggested that "the use of catalase activity as an index of adequacy of scald, particularly when the less reliable qualitative methods are used, is not a generally reliable method."

Mergentime *(20)* and Balls *(2)* suggested that peroxidase activity might serve as an index of adequacy of heating. In a review paper, Joslyn *(11)* refers to earlier work in which it was found that peroxidase activity more closely paralleled the formation of off-flavors in frozen vegetables than did catalase activity. However, he cautions that the correlation varied markedly with nature of the substrate used for detecting peroxidase activity.

The first study quantitatively relating degree of peroxidase inactivation to quality retention in frozen vegetables was that of Masure and Campbell *(18, 28)*. In this study, rapid methods were devised for the quantitative and semiquantitative estimation of peroxidase in various vegetable tissues. The latter method was developed for quality control in processing plants. These tests are based on an observed relationship between time required for appearance of color produced by the residual peroxidase in a filtered extract of vegetable in a guaiacol and hydrogen-peroxide substrate, on the one hand, and retention of quality in the frozen product on the other.

Specifications for the semiquantitative test were based on correlation

of degree of heating with retention of quality as evaluated by panel for several vegetables stored 4 years at –5°F. (–20.6°C.). In these studies, it was shown that samples heated for a time sufficient for quality retention during freezing storage required 3.5 minutes for color development in the test. These same samples were shown to contain 1 or 2% of residual peroxidase, as determined by the quantitative test. Thus, the semiquantitative test sets a safe minimum of time required to inactivate 98 to 99% of the peroxidase in the vegetable. A trained technologist can apply the semiquantitative test in a plant quickly and routinely. Careful attention to procedure and reagents will assure reliable results, when the test is applied to material a few minutes after heating and cooling.

Although peroxidase inactivation as an index of adequacy seems well established for many vegetables, the catalase index is still used by many processors. In view of this fact, the present study was undertaken to compare degrees of inactivation of these two enzymes with degrees of quality retention in frozen peas stored over a range of temperatures. The factors considered were color, flavor, and skin texture as determined by organoleptic evaluation, and ascorbic acid content, chlorophyll degradation, and reflectance color as determined by objective measurements.

PREPARATION OF SAMPLES

Samples were prepared from raw material (Dark Skin Perfection variety) from a commercial processing line just preceding the plant blancher. The field-run tenderometer value was 104. Sufficient material for the entire experiment was thoroughly mixed to insure uniformity and then heavily iced during the time required for preparation of experimental lots. For each variable, a 30-pound lot was used. This lot was placed in a perforated basket and the temperature of the peas was adjusted to 68°F. (20.0°C.) by submergence in water. Scalding was carried out in boiling water that was continuously heated in a steam-jacketed kettle. The basket was raised and lowered continuously to insure uniform heating. The temperature of the water varied from 208°F. (97.8°C.) initially to 192°F. (88.9°C.) after 40 seconds, then to 200°F. (93.3°C.) after 90 seconds, and 206°F. (96.7°C.) after 160 seconds. The peas were cooled immediately by submersion and agitation in running cold water for 2 minutes.

The samples were packed in 10-oz. wax-paper cartons, plate frozen, and then overwrapped with wax paper. Maximum time between blanching and freezing was 1.5 hours. Peroxidase activity of all samples was determined within a few minutes after blanching by the semiquantitative test *(28)*.

The materials were shipped to the Laboratory under dry ice. All samples were placed in storage at –10°F. (–23.3°C.) for 1 month. During this period enzyme assays were carried out. Subjective evaluations were made after 1 month and samples were selected for further storage at –10°F. (–23.3°C.) and storage at 0°F. (–17.8°C.) and 10°F. (–12.2°C.). Control materials were held at –30°F. (–34.4°C.).

CHEMICAL AND PHYSICAL ANALYSES

Peroxidase. Determinations were made by the semiquantitative and quantitative methods of Masure and Campbell *(18, 28)* and by the titrimetric procedure of Morris, Weast, and Lineweaver *(21)* Table 1. In the former, peroxidase activity is measured by color development in a substrate of guaiacol and hydrogen peroxide and in the latter by rate of disappearance of hydrogen peroxide, with guaiacol as substrate.

The semiquantitative test conducted shortly after scalding showed that samples heated 70 seconds were slightly underheated while those blanched 90 seconds were adequately blanched. Examination at later storage intervals revealed increased peroxidase activity in lots heated 90 seconds or less. These results agree with those obtained in unpublished work carried out in this Laboratory by M. P. Masure and H. S. Olcott

TABLE 1

Peroxidase assays

Storage temp. (°F.)	Storage period (months)	Blanching time (sec.)					
		20	35	50	70	90	180
		Semiquantitative test (18), sec. for appear. of color[1]					
–	0[2]	–	30	60	180	780	>45 min.
–10	¾	3	3	15	30	120	>45 min.
	7	10	17	63	122	180	>45 min.
	13	5	7	24	52	168	>10 min.
0	6[3]	8	23	45	91	182	>10 min.
	12	5	8	21	45	115	>10 min.
10	6[3]	12	25	32	94	178	>10 min.
	12	6	7	15	43	105	>10 min.
		Quantitative test (18), D/M units					
–10	¾	404[4]	303	165	58	26	0
	7	377	307	174	62	25	0
	13	402	276	160	84	23	0
0	6[3]	350	317	160	74	28	0
	12	430	302	176	70	31	0
10	6[3]	359	292	193	78	30	0
	12	339	293	194	78	30	0
Average value		380	297	175	72	28	0
Per cent residual		92	72	42	17	7	0
		Quantitative, titrimetric method (21), moles of hydrogen peroxide used per minute per gram tissue; data × 10^7					
–10	7	452	383	218	101	42	0
	13	537	437	238	87	37	0
0	6	527	414	230	94	41	0
	12	588	475	275	87	50	0
10	6	472	420	206	82	42	0
	12	562	413	225	100	50	0
Average value		523	424	232	92	44	0

[1] Two hundred and ten seconds is the time set by the test for adequate blanching.
[2] Determinations made a few minutes after blanching.
[3] These materials had been stored at –10°F. for one month prior to being placed at the elevated temperature.
[4] Raw sample, 414 units.

in which underblanched peas or peas heated to "borderline" inactivation as noted by the semiquantitative test showed increased peroxidase activity when allowed to stand at room temperature for 1 to 2 hours before freezing. Since the present samples were necessarily delayed about 1.5 hours between scalding and freezing, the changes in peroxidase activity noted most likely occurred during this delay.[5]

As is shown under "Organoleptic Evaluations," the material blanched 70 seconds, although it contained 17% residual peroxidase as determined 1 month after preparation, showed no significant change in quality which could be attributed specifically to the residual enzyme content. Thus, this test not only serves as an index of adequacy

[5] A delay of this magnitude is far in excess of that which would be encountered under conditions of good commercial practice.

of blanching in frozen peas but offers a considerable margin of safety. These conclusions were borne out by previous unpublished work on peas carried out in this Laboratory and in similar studies on Brussels sprouts *(19)*.

The differences in enzyme activity noted at the various examination periods are not considered outside the range to be expected as a result of differences due to sampling and inherent sources of error in the assay methods.

Catalase. Qualitative assay for catalase was made by the fermentation tube method used by many members of the freezing industry. This is a modification of the method described by Proctor *(23)*. In the test, a known weight of sample plus approximately one-half the weight of calcium carbonate is suspended in a small measured amount of water by grinding with sand and calcium carbonate in a mortar. The water and suspended, ground materials are transferred to a fermentation tube and mixed with a known volume of 3% hydrogen peroxide. Care is taken to avoid occlusion of air. The tube is shaken gently for 3 minutes. If the volume of gas present is greater than that observed when a cooked sample is treated similarly, the test is considered positive. Quantitative assay was made by the method of Lineweaver and Morris *(15)*. The activities by this method are expressed in K_f units (Table 2).

TABLE 2

Catalase assays

Storage temp. (°F.)	Storage period (months)	Blanching time (sec.)					
		20	35	50	20	35	50
		Quantitative method *(18)*, K_f values			Qualitative method *(23)*, inches of gas in fermentation tube		
−10	¾	0.119	0.006	0	1.3	0.00	0
	7	0.126	0.005	0	2.4	0.04	0
	13	0.136	0.004	0	2.2	0.04	0
0	6[1]	0.123	0.006	0	3.4	0.04	0
	12	0.121	0.004	0	2.6	0.04	0
10	6[1]	0.108	0.004	0	2.7	0.04	0
	12	0.096	0.005	0	2.0	0.04	0
Average value		0.118	0.005	0	–	–	–
Per cent residual		24[2]	1	0	–	–	–

[1] See footnote 4, Table 1.
[2] K_f value on frozen raw sample after 3 weeks' storage at −10°F. (−23.3°C.), 0.492.

In samples blanched 35 seconds, approximately 99% of the catalase present was inactivated. Thus, 50 seconds would be considered more than adequate if catalase inactivation is used as the index. However, as is shown under "Organoleptic Evaluation" these samples showed a significant deterioration in flavor after 6 months' storage at −10°F. (−23.3°C.).

No significant change in residual enzyme content was noted in the samples during the 12-month storage interval.

Chlorophyll. The conversion of chlorophyll to pheophytin, which commonly occurs during processing of green vegetables, is a good index of color deterioration and is correlated closely with subjective evaluation of color *(3, 7)*. The effects of degree of blanching and storage temperature on this conversion has been investigated by a modification of the method of Dutton, Bailey, and Kohake *(7)*. An acetone extract of peas was prepared by blending 140 grams of the frozen material with 100 ml. of acetone for 3 minutes. Eighty grams of slurry were diluted with 300 ml. of acetone. After stirring for 3 minutes, the mixture was filtered with gentle suction through a coarse sintered-glass filter. The first 100-ml. portion of filtrate, which is generally somewhat cloudy, was refiltered. The residue was washed twice by mixing with 50-ml. portions of acetone and the combined filtrates were diluted to 500 ml. with 80% acetone. The optical density of the solution was determined at 534 and 556 mμ with a Beckmann Model DU spectrophotometer and the ratio of chlorophyll to pheophytin determined by the method of Mackinney and Weast *(17)* (Figure 1).

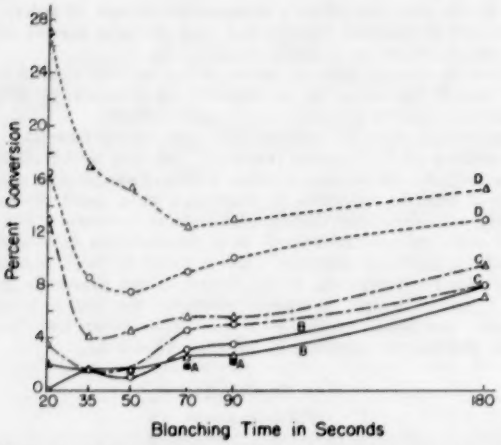

Figure 1. Effect of blanching time and storage time on conversion of chlorophyll to pheophytin in frozen peas. Each of the two filled squares labeled A represents an average of percentages at 6 and 12 months, with storage at −30°F. Curves labeled B represent −10°F.; C, 0°F.; D, 10°F. Circles represent percentages at 6 months, triangles at 12 months.

The results show that rate and extent of chlorophyll degradation are affected by both degree of heating and storage temperature. The greatest change was observed in the samples blanched for shorter times and stored at higher temperatures.

Wagenknecht, Lee, and Boyle *(26)* reported a loss in chlorophyll in raw peas held 1 and 5 years at 0°F. (−17.8°C.), while blanched peas from the same lot stored under the same conditions showed little change. They postulated that the enzymes lipoxidase and lipase and a large increase in acid number accounted for chlorophyll breakdown of the raw peas. Campbell *(4)* considered the loss of green color observed in frozen Alderman peas which were adequately blanched but stored at insufficiently low temperatures [15° and 20°F. (−9.4° and −6.7°)] to be due to the slow transformation of chlorophyll to pheophytin by the action of the acids of the cell sap.

In the light of these conclusions, the results obtained in the present work suggest that the chlorophyll breakdown observed in the samples blanched 20 seconds, which contain substantial amounts of active enzymes, is probably in large part enzymatic in nature. As the blanching times are increased, and the enzymes progressively inactivated, it would be expected that conversion due to chemical action would predominate. In materials stored at −10°F. (−23.3°C.), there was a gradual increase in conversion with increasing time of blanch to about 8% in the 180-second-blanched sample. These changes undoubtedly occurred during blanching and packaging, since the values for each blanching time remain essentially unchanged during storage. Similar observations were made by Talburt and Legault *(25)*.

Reflectance color. Measurements to determine color differences between samples were made with a Hunter Color and Color-Difference Meter *(10, 13)*. The reference standard employed throughout was a tile with values of Rd, *a*, and *b* of 31.0, −24.0, and +13.1, respectively, visually matched to the National Bureau of Standards (NBS) master tile No. SBC-12.

The frozen material was placed in polyethylene bags and thawed by immersion in tap water at 90°F. (32.2°C.). After thawing, the material was transferred to a cell 3.26 inches in diameter by 1 inch deep. Sufficient distilled water was added to fill the cell to a height of 0.5 to 0.75 inch. Measurements were made through the bottom of the cell. A large opening (2.25 inches in diameter) in the cover plate on the instrument was used. Each value of Rd, *a*, and *b*, reported is an average of 3 readings on a single sample mixed between readings.

Rd values were converted to L (lightness) values to provide a grey scale uniform in visual differences. Total color difference, ΔE, was calculated according to the formula, $\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$ in which ΔL, Δa, and Δb are differences in values between the selected control sample and each experimental sample. The control samples were taken from the 70-second-blanched lot which had been held at −30°F. (−34.4°C.). The data are presented in Table 3.

TABLE 3

Hunter color difference meter measurements and calculated total color difference (ΔE)

Storage temp. (°F.)	Blanch time (sec.)	Storage time: 6 months Rd	L	a	b	ΔE	12 months Rd	L	a	b	ΔE
−30	70	10.3	39.6	−19.1	+16.8	0.4	10.3	39.6	−18.3	+16.7	0.4
−30	90	10.4	39.7	−18.6	+17.1	0.4	10.2	39.4	−18.6	+16.7	0.3
Mean value of −30° samples		10.3	39.6	−18.7	+16.7						
−10	20	9.1	37.6	−17.2	+15.5	2.8	8.8	37.0	−15.2	+14.9	4.7
	35	9.4	38.0	−17.1	+16.0	2.4	8.8	37.0	−16.6	+15.2	3.7
	50	9.4	38.0	−17.6	+16.0	2.1	8.8	37.0	−17.4	+15.4	3.2
	70	10.1	39.2	−18.4	+17.2	0.7	9.4	38.0	−17.0	+15.6	2.6
	90	10.3	39.6	−18.6	+17.5	0.8	9.3	37.9	−17.6	+16.3	2.1
	180	10.4	39.7	−18.2	+17.3	0.8	10.0	39.1	−17.4	+16.7	1.4
0	20	9.1	37.6	−16.1	+15.3	3.6	9.1	37.6	−15.8	+15.1	3.9
	35	9.3	37.9	−17.5	+15.8	2.3	9.1	37.6	−15.8	+15.1	3.9
	50	9.4	38.0	−18.0	+16.2	1.8	9.5	38.2	−16.8	+15.4	2.7
	70	10.1	39.2	−18.6	+16.9	0.5	10.0	39.1	−17.6	+16.6	1.2
	90	10.3	39.6	−18.6	+17.2	0.5	9.7	38.6	−16.9	+16.4	2.1
	180	10.4	39.7	−18.5	+17.2	0.5	9.9	39.0	−16.9	+16.4	1.9
10	20	8.2	35.8	−13.9	+13.5	6.8	9.1	37.6	−13.2	+15.2	6.0
	35	8.1	35.6	−15.1	+13.6	5.5	9.0	37.4	−15.2	+15.6	4.3
	50	8.0	35.4	−15.6	+14.0	5.9	8.9	37.2	−15.3	+15.3	4.4
	70	9.1	37.6	−16.3	+14.8	3.7	9.3	37.9	−16.1	+15.8	3.2
	90	8.8	39.0	−15.7	+14.8	4.4	9.3	37.9	−16.2	+16.0	3.1
	180	9.0	37.4	−16.1	+15.0	3.8	9.7	38.6	−16.4	+16.3	2.5

The color value L indicates lightness and the values a and b locate points on Hunter's chromaticity graph. From the location of a point an indication of the hue and saturation of the color can be obtained. An examination of the data suggests dividing the samples into three groups: (a) those blanched for the shorter times, 20, 35, and 50 seconds, and stored at −10° and 0°F.; (b) those blanched for the longer times, 70, 90, and 180 seconds, and stored at −10° and 0°F.; and (c) the entire series stored at 10°F. Throughout storage, group (a) showed a general loss of saturation in the dominant hue (yellow-green) as shown by changes in values of a and b and a diminishing lightness of the overall color as determined by the changes in L values. As blanching time was increased, the changes became smaller.

The 6-month changes in group (b) were about the same order of magnitude as the precision of measurement. At the 12-months examination, these samples showed a decrease in saturation with a resultant loss in lightness. The materials in group (c) showed the greatest change in color. During the first 6 months, an increase of greyness (loss of saturation) occurred. On further storage there was a change toward the yellow with an increase in lightness of the overall color. The greatest changes were noted in samples blanched 20 and 35 seconds.

The values of ΔE are expressed in NBS units of color difference which are intended to be of such size that measured differences of less than one unit would

represent perceptually unimportant color differences, as described by Eastmond *et al. (8)*. The values vary both with blanching time and storage temperature. Samples inadequately blanched (20, 35, and 50 seconds) showed the greatest change in ΔE values. Changes in samples stored at −10° and 0°F. were similar in magnitude. Storage at 10°F. superimposed an additional change in ΔE values up to 4 NBS units over that caused by inadequate blanching and storage at lower temperatures. The greatest degree of change in samples was noted during the first 6 months of storage.

Ascorbic acid. Determinations of reduced ascorbic acid content of all samples were carried out by the method of Loeffler and Ponting *(16)* as modified by Ponting *(22)*. In the analyses, 130 grams of peas were blended with 5 times their weight of 0.5% oxalic acid solution. After blending, 6 grams of analytical filter-aid were added and, after stirring, the mixture was filtered. Triplicate determinations of ascorbic acid were made on the filtrate by the colorimetric procedure. The data are shown in Figure 2.

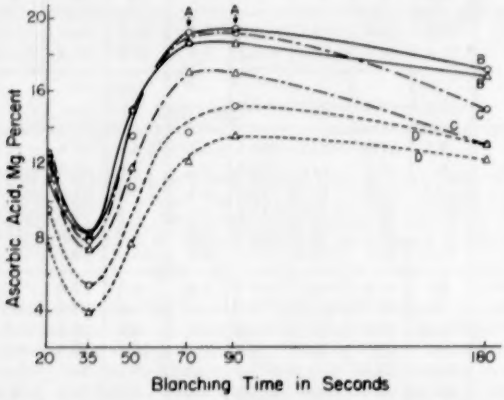

Figure 2. Effect of blanching time and storage time on retention of ascorbic acid in frozen peas. Percentages for −30°F. (designated A) are averages for 6 and 12 months and are the same as those for −10°F. (designated B). Other curves are identified in legend for Figure 1.

Facilities were not available at the time of processing to allow for the determination of ascorbic acid content of the raw peas. Thus, the samples stored one month at −10°F. (−23.3°C.) and then transferred to −30°F. (−34.4°C.) during subsequent storage were used for reference standards. On the basis of previous studies *(14)*, the changes in ascorbic acid which would be expected to occur after one month of storage at −10°F. (−23.3°C.) would be negligible, while those occurring at −30°F. (−34.4°C.) would be within the limit of error of the determination. These values would be expected to be lower than the true raw material by the amount of ascorbic acid lost by leaching and enzyme action during the blanching operation.

It will be noted that samples blanched to a degree sufficient to inactivate peroxidase, as indicated by the semiquantitative method carried out at time of blanching, showed the greatest retention of ascorbic acid. On the other hand, those blanched for shorter times (50, 35, and 20 seconds) and containing increased amounts of active enzymes showed a considerable loss of ascorbic acid during processing. This is probably due in large part to enzymatic action. Such loss has been discussed in some detail by Hartzler and Guerrant *(9)*. Storage of all samples at higher temperatures brought about further losses in ascorbic acid and the magnitude of which increased with storage time and temperature.

ORGANOLEPTIC EVALUATIONS

The initial evaluation of materials was made after one month of storage at −10°F. (−23.3°C.). Samples from each of the 6 blanching times, cooked according to our

general procedure,[b] were presented to a panel of 11 judges at 4 grading sessions. Thus, 44 judgments were obtained on each of the samples for the factors considered, namely, color, flavor, and skin texture. The analysis of variance and the Least Significant Differences (LSD) were calculated from the judges' mean scores for each sample. In the case of the latter values (LSD), the calculations were carried out by the method of Scheffé *(24)* as adapted by Wood, Nutting, and Olson *(27)*. The mean scores and LSD's are given in Table 4.

TABLE 4

Panel evaluation after 1 month storage at −10°F. ("zero" appraisal)

Blanching time (sec.)	Mean scores[1]		
	Color	Flavor	Skin texture
20	4.3	2.5	3.4
35	4.7	3.5	3.7
50	4.6	4.0	3.6
70	4.6	4.0	3.7
90	4.4	4.1	3.8
180	4.5	4.2	4.0
LSD for P = 0.05	N.S.	1.0	0.5

[1] Scores on 7-1 basis.

Color 7 = Bright green
1 = Gray or yellow green
Flavor 7 = No off-flavor
1 = Much off-flavor
Skin Texture
7 = Very tender
1 = Very tough

The data showed that the sample blanched 20 seconds had a significantly lower flavor score than other samples and had significantly tougher skins than the 180-second-blanched sample.

After initial appraisal, samples blanched 50, 70, 90, and 180 seconds were placed in storage at −10°, 0°, and 10°F. (−23.3°, −17.8°, and −12.2°C.) for subsequent examination. These samples, judged on the basis of the semiquantitative peroxidase test carried out at the time of blanching, were considered underblanched, nearly adequately blanched, adequately blanched, and twice adequately blanched, respectively. In addition, samples blanched 70 and 90 seconds were placed in storage at −30°F. (−34.4°C.) as control material to be used in the later evaluations of samples stored at −10°, 0°, and 10°F. (−23.3°, −17.8°, and −12.2°C.).

After 6 and 12 months of storage, the samples were evaluated by a panel of 12 to 14 judges. The group of samples examined at each period was composed of material from the 4 blanching lots stored at each of the 3 temperatures plus a hidden control [the 70-second-blanched sample stored at −30°F. (−34.4°C.)]. Because of the large number of samples to be evaluated, the balanced incomplete block design for 13 samples, plan 11.22 of Cochran and Cox *(5)*, was employed. In the plan 4 coded samples and a labeled control were presented at each of 13 judging periods. During the examination period, each sample was judged 4 times and was compared once against each of the other samples.

Flavor and skin texture were judged together. Green lights were used in the taste booths to mask any color differences between samples. Color was judged on another set of samples which was presented at the same judging period. Ordinary incandescent lamps equipped with daylight filters were used as the source of illumination. The data are presented in Table 5.

Flavor data show that, with one exception, the sample blanched 50 seconds (which contained no active catalase but 40 to 50% residual peroxidase) was graded lower

[b] A given weight of frozen peas was introduced into one-half the weight of boiling water. After the water had again come to a boil, cooking was continued for 5 minutes.

TABLE 5

Panel evaluation after 6 and 12 months storage

Storage temp. (°F.)	Blanching time (sec.)	Mean scores[1]					
		6 months			12 months		
		Color	Flavor	Skin tex.	Color	Flavor	Skin tex.
–30	(Control) 70	4.6	4.4	4.3	4.6	4.6	4.8
–10	50	4.5	3.3	4.0	4.7	3.3	4.7
	70	4.8	4.1	4.3	4.8	4.2	4.7
	90	4.5	4.4	4.3	4.5	4.3	4.7
	180	4.5	4.3	4.4	4.2	4.0	4.8
0	50	4.6	3.0	4.3	4.6	3.2	4.6
	70	4.8	4.0	4.5	4.3	3.5	4.7
	90	4.8	4.2	4.2	4.3	3.8	4.6
	180	4.4	4.2	4.6	4.0	3.4	4.6
10	50	3.8	3.2	4.1	3.2	3.0	4.5
	70	4.2	3.1	4.1	3.5	3.6	4.5
	90	4.5	3.6	4.3	3.5	3.3	4.4
	180	4.2	3.5	4.2	3.2	3.4	4.5
LSD for P = 0.05		0.6	1.1	0.6	0.6	1.0	0.6

[1] Scoring on a 7-1 basis as shown in Table 4. Labeled control presented at each judging period was assigned score 5.

than all of the other samples and in all instances was graded significantly lower than the control sample. At both the 6- and 12-month storage intervals there was a definite trend toward lower flavor scores in samples stored at 10°F. (–12.2°C.). A similar trend was noted in samples stored at 0°F. (–17.8°C.) for 12 months. These results are in general agreement with earlier work *(14)*.

The data clearly indicate the effect of degree of enzyme inactivation and storage temperature on development of off-flavor in frozen peas.

Significant differences in visual color between the control and the experimental samples were noted by the panel in all samples stored at 10°F. (–12.2°C.) for 12 months. In addition, the sample blanched 50 seconds and stored at 10°F. for 6 months and the sample blanched 180 seconds and stored at 0°F. (–17.8°C.) for 12 months showed visual color change. In all of these samples, a 7 to 15% conversion of chlorophyll to pheophytin occurred. Skin texture was essentially unaffected by either blanching time or storage temperature.

DISCUSSION

The relative ease of inactivation of catalase by heat suggests that samples containing active catalase probably contain other active enzyme systems which would play a role in the destruction of ascorbic acid and the degradation of chlorophyll. The magnitude of changes observed as affected by storage temperature and time also suggests the presence of an active biological catalyst in the samples blanched for the shorter times. These observations are in general agreement with previous work *(9, 26)*.

Although heating to a point sufficient to inactivate catalase was sufficient for chlorophyll and ascorbic acid retention in samples stored under conditions that would be considered good commercial practice, namely, –10° and 0°F. (–23.3° and –17.8°C.), it was not sufficient for flavor retention. This fact is evidenced by the sample blanched 50 seconds, which contained no active catalase but contained 40 to 50% residual peroxidase.

It received lower flavor scores than all of the other samples judged, even when stored at –10°F. This finding agrees with the report of Joslyn *(11)* in which he stated that peroxidase activity paralleled the formation of off-flavors in vegetables more closely than catalase activity.

Samples blanched just adequately to the semiquantitative peroxidase test *(28)* were better in flavor and color and contained a greater amount of ascorbic acid than samples blanched for longer or shorter times. They retained excellent quality when stored for 1 year at –10° F., and for at least 6 months when stored at 0°F., while materials stored at 10°F. showed some deterioration after 6 months' storage and a marked deterioration after 1 year.

Two points should be emphasized in connection with these studies. The first is concerned with a question often asked by people interested in frozen food: "How long should a sample be blanched in order to retain satisfactory quality during storage?" It is obvious that specific times cannot be suggested, since the time required to inactivate the enzyme systems responsible for quality deterioration is a function of many factors. Some examples are temperature of the blancher, temperature of material entering the blancher, load of material going through the blancher, and rapidity of cooling after blanching. Thus the blanching time required is the time necessary for the inactivation of an enzyme system which has been found to serve as an index of adequacy for frozen vegetables. The present study, as well as previous work, has shown that inactivation of peroxidase to the end-point of the semiquantitative test meets this requirement. Peas blanched to this end-point have retained good quality during freezing storage.

The second point is concerned with interpretation of the results of organoleptic evaluation of the samples. These data were obtained with the aid of well trained judges and the changes noted represent what might be termed "first detectable changes" in the samples. Although it is not expected that these changes are sufficient to cause consumer complaint, they are nevertheless important, since they emphasize the lability of products when subjected to improper processing and storage condiions.

In the channels of commerce exposure of frozen commodities to temperatures well above those used in the present study is not uncommon. Work now in progress in this Laboratory indicates that the effects of intermittent exposure to higher temperatures are essentially additive. Since the undesirable effects of under-blanching would be added to the deteriorative effects of storage, the need for proper blanching and storage for best quality retention is apparent.

SUMMARY

The data presented clearly indicate the effect of degree of catalase and peroxidase inactivation and storage temperature on quality of frozen peas. Materials containing active catalase showed greater loss of ascorbic acid and degradation of chlorophyll than materials in which catalase and peroxidase had been inactivated. The sample of peas blanched just adequately for a negative semiquantitative peroxidase test maintained better flavor, color, and more ascorbic acid than the samples blanched for shorter or longer times. These peas retained excellent quality for 1 year. In all

instances, the magnitude of changes was found to increase with increasing storage temperature.

Acknowledgment

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A METHOD OF CHOOSING JUDGES FOR A SENSORY EXPERIMENT

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A method for choosing a panel of judges for a sensory experiment to insure that the individual judges and the panel as a whole can detect differences at any given probability level is described in this article. Use is made of a study by D. W. Chapman[a] in which the following problems were solved: (a) What is the probability that a single chance arrangement of two series of t elements each will result in exactly s correct matchings? (b) What is the probability that a single chance arrangement of the two series will result in at least s correct matchings? (c) What is the probability that the mean number of correct matchings for n independent chance arrangements of the two series will be at least $\bar{s}$?

Chapman has tabulated the probabilities of obtaining exactly s correct matchings for all values of t and s. It happened that the probabilities associated with any value of s converge so rapidly that actual calculation beyond $t = 9$ is unnecessary. The formula used to compute these probabilities is given by (i).

$$\text{(i)} \quad P(s) = \frac{1}{s!}\left[\frac{1}{0!} - \frac{1}{1!} + \frac{1}{2!} - \frac{1}{3!} + \ldots + (-1)^{t-s}\frac{1}{(t-s)!}\right]$$

Chapman has also tabulated the probability of obtaining s or more correct matchings when t things are arranged. This table is necessary since the real test of significance of an obtained s is the probability of obtaining by chance a value of s as great or greater. This table[b] is given below.

TABLE 1

The probability of obtaining s or more correct matchings when t things are arranged

t	s → o	1	2	3	4	5	6	7	8	9	10
1	1.0000	1.0000									
2	1.0000	.5000	.5000								
3	1.0000	.6667	.1667	.1667							
4	1.0000	.6250	.2917	.0417	.0417						
5	1.0000	.6333	.2583	.0916	.0083	.0083					
6	1.0000	.6319	.2652	.0777	.0221	.0014	.0014				
7	1.0000	.6321	.2641	.0808	.0183	.0044	.0002	.0002		*All further*	
8	1.0000	.6321	.2642	.0802	.0191	.0035	.0007	.0000	.0000	*terms approx-*	
$t \geqq 9$	1.0000	.6321	.2642	.0803	.0190	.0037	.0006	.0001	.0000	*imate* .0000	

[a] Reference is made to D. W. Chapman, the statistics and methods of correct matchings, *Am. J. Psychol.*, **46**, 287-298 (1934). Permission to quote appropriate sections of this article was kindly granted by the editor of The American Journal of Psychology in a letter to the author, 4 April 1955.

[b] Chapman's Table II.

Chapman defined the mean number of correct matchings for n independent trials by (ii).

$$\text{(ii)} \quad \bar{s} = \frac{s_1 + s_2 + \ldots + s_n}{n}$$

He also gives a table[c] showing the probability up to $n = 30$ that the mean number of correct matchings resulting from n independent trials will be as great as $\bar{s}$ or greater, when $t \geqq 4$. Values are given below for only $n = 2, 3, 4$ and 5.

TABLE 2

Showing the probability (p) that the mean number of correct matchings resulting from n independent trials will be as great as $\bar{s}$ or greater, when $t \geqq 4$

n = 2		n = 3		n = 4		n = 5	
$\bar{s}$	p	$\bar{s}$	p	$\bar{s}$	p	$\bar{s}$	p
1.000	.597	1.000	.580	1.000	.568	1.000	.559
1.500	.325	1.333	.354	1.250	.371	1.200	.386
2.000	.143	1.667	.186	1.500	.215	1.400	.134
2.500	.054	2.000	.085	1.750	.111	1.600	.068
3.000	.018	2.333	.035	2.000	.052	1.800	.033
3.500	.005	2.667	.013	2.250	.022	2.000	.015
4.000	.002	3.000	.004	2.500	.009	2.200	.006
4.500	.000	3.333	.001	2.750	.003	2.400	.002
		3.667	.000	3.000	.001	2.600	.001
				3.250	.000	2.800	.000

The results that Chapman obtained have a direct application in choosing judges for a sensory experiment.

Consider that we wish to conduct an experiment to determine if there is any difference in the saltiness of meat cured by five different processes. The differences in the saltiness of the meat will be very slight; therefore, we must be sure that the judges are able to discriminate between very slight differences.

Let us assume that we require five judges for our experiment and we would like to have the individual judges as well as the panel as a whole be able to detect differences at the 10% level. As a method of choosing our judges, solutions of salt varying in concentration could be prepared in duplicate. The potential judges would then be asked to match the identical solutions and the number of correct matchings recorded.

As an example consider the following:

TABLE 3

Exemplifying the method, by hypothetical results for $t = 5$

Individual	1	2	3	4	5	6	7	8	9	10
s	2	1	0	3	4	0	5	4	3	2
P (s or more)	.2583	.6333	1.0000	.0916	.0083	1.0000	.0083	.0083	.0916	.2583

[c] Chapman's Table III.

It is seen that we can obtain five judges who can detect differences in saltiness at the 10% level by choosing individuals 4, 5, 7, 8, and 9. In order to see if these individuals acting as a panel could detect differences at the 10% level we must calculate $\bar{s}$.

$$\bar{s} = \frac{3 + 4 + 5 + 4 + 3}{5} = \frac{19}{5} = 3.8$$

By looking at Table 2 we see that the probability associated with 3.8 is of the order of .000.

In the above example, we considered that each individual tried to match the samples only once, in practice they could be asked to repeat this operation as many times as we choose. Chapman actually has tabulated the probabilities of obtaining as great as $\bar{s}$ or greater for $t \geqq 4$ and $n = 2, 3, \ldots, 15, 20, 25$, and 30.

If fatigue is going to be a problem, the number of duplicate samples could be reduced. If fatigue is not a problem, the number of duplicate samples could be increased.

CHAPMAN'S COMPLETE TABLES

For the convenience of research workers engaged in sensory testing, Chapman's complete tables[4] are reproduced:

TABLE I

The probability of obtaining exactly s correct matchings when t things are arranged

t	s → o	1	2	3	4	5	6	7	8	9	10
1	.0000	1.0000									
2	.5000	.0000	.5000								
3	.3333	.5000	.0000	.1667							
4	.3750	.3333	.2500	.0000	.0417						
5	.3667	.3750	.1667	.0833	.0000	.0083					
6	.3681	.3667	.1875	.0556	.0208	.0000	.0014				
7	.3679	.3681	.1833	.0625	.0139	.0042	.0000	.0002		*All further*	
8	.3679	.3679	.1840	.0611	.0156	.0028	.0007	.0000	.0000	*terms approx-*	
$t \geqq 9$	.3679	.3679	.1839	.0613	.0153	.0031	.0005	.0001	.0000	*imate* .0000	

TABLE II

The probability of obtaining s or more correct matchings when t things are arranged

t	s → o	1	2	3	4	5	6	7	8	9	10
1	1.0000	1.0000									
2	1.0000	.5000	.5000								
3	1.0000	.6667	.1667	.1667							
4	1.0000	.6250	.2917	.0417	.0417						
5	1.0000	.6333	.2583	.0916	.0083	.0083					
6	1.0000	.6319	.2652	.0777	.0221	.0014	.0014				
7	1.0000	.6321	.2641	.0808	.0183	.0044	.0002	.0002		*All further*	
8	1.0000	.6321	.2642	.0802	.0191	.0035	.0007	.0000	.0000	*terms approx-*	
$t \geqq 9$	1.0000	.6321	.2642	.0803	.0190	.0037	.0006	.0001	.0000	*imate* .0000	

[4] Permission for reproducing these tables was granted by the editor, *Am. J. Psychol.*; see footnote a.

TABLE III

Showing the probability (p) that the mean number of correct matchings resulting from n independent trials will be as great as $\bar{s}$ or greater, when $t \geqq 4$

n = 2		n = 3		n = 4		n = 5	
$\bar{s}$	p	$\bar{s}$	p	$\bar{s}$	p	$\bar{s}$	p
1.000	.597	1.000	.580	1.000	.568	1.000	.559
1.500	.325	1.333	.354	1.250	.371	1.200	.386
2.000	.143	1.667	.186	1.500	.215	1.400	.134
2.500	.054	2.000	.085	1.750	.111	1.600	.068
3.000	.018	2.333	.035	2.000	.052	1.800	.033
3.500	.005	2.667	.013	2.250	.022	2.000	.015
4.000	.002	3.000	.004	2.500	.009	2.200	.006
4.500	.000	3.333	.001	2.750	.003	2.400	.002
		3.667	.000	3.000	.001	2.600	.001
				3.250	.000	2.800	.000

n = 6		n = 7		n = 8		n = 9	
$\bar{s}$	p	$\bar{s}$	p	$\bar{s}$	p	$\bar{s}$	p
1.000	.553	1.000	.551	1.000	.549	1.000	.546
1.167	.395	1.143	.401	1.125	.408	1.111	.412
1.333	.257	1.286	.270	1.250	.284	1.222	.294
1.500	.153	1.429	.171	1.375	.185	1.333	.198
1.667	.085	1.571	.099	1.500	.112	1.444	.124
1.833	.043	1.714	.054	1.625	.064	1.556	.074
2.000	.021	1.857	.028	1.750	.035	1.667	.042
2.167	.010	2.000	.013	1.875	.018	1.778	.032
2.333	.004	2.143	.006	2.000	.009	1.889	.011
2.500	.001	2.286	.003	2.125	.004	2.000	.006
2.667	.000	2.429	.001	2.250	.002	2.111	.003
		2.571	.000	2.375	.001	2.222	.001+
				2.500	.000	2.333	.001−
						2.444	.000

n = 10		n = 11		n = 12		n = 13	
$\bar{s}$	p	$\bar{s}$	p	$\bar{s}$	p	$\bar{s}$	p
1.000	.543	1.000	.540	1.000	.537	1.000	.536
1.100	.416	1.091	.421	1.083	.426	1.077	.427
1.200	.304	1.182	.312	1.167	.419	1.154	.323
1.300	.209	1.273	.220	1.250	.228	1.231	.237
1.400	.135	1.364	.145	1.333	.156	1.308	.165
1.500	.084	1.455	.092	1.416	.101	1.385	.110
1.600	.049	1.545	.056	1.500	.063	1.462	.070
1.700	.027	1.636	.033	1.583	.038	1.538	.043
1.800	.015	1.727	.018	1.667	.022	1.615	.026
1.900	.008	1.818	.010	1.750	.012	1.692	.014
2.000	.004	1.909	.005	1.833	.006	1.769	.008
2.100	.002	2.000	.002	1.916	.003	1.846	.004
2.200	.001	2.091	.001+	2.000	.002	1.923	.002
2.300	.000	2.128	.001−	2.083	.001	2.000	.001+
		2.273	.000	2.167	.000	2.077	.001−
						2.154	.000

TABLE III (concluded)

n = 14		n = 15		n = 20		n = 25		n = 30	
$\bar{s}$	p	$\bar{s}$	p	$\bar{s}$	p	$\bar{s}$	p	$\bar{s}$	p
1.000	.533	1.000	.534	1.000	.529	1.000	.527	1.000	.524
1.071	.431	1.067	.432	1.050	.445	1.040	.447	1.033	.452
1.143	.331	1.133	.335	1.100	.355	1.080	.371	1.067	.384
1.214	.243	1.200	.251	1.150	.279	1.120	.300	1.100	.314
1.286	.173	1.267	.181	1.200	.213	1.160	.237	1.133	.255
1.357	.118	1.333	.125	1.250	.156	1.200	.182	1.167	.203
1.429	.077	1.400	.083	1.300	.113	1.240	.137	1.200	.158
1.500	.048	1.467	.053	1.350	.079	1.280	.101	1.233	.119
1.571	.029	1.533	.037	1.400	.052	1.320	.072	1.267	.089
1.643	.017	1.600	.020	1.450	.035	1.360	.050	1.300	.065
1.714	.010	1.667	.011	1.500	.022	1.400	.034	1.333	.047
1.786	.005	1.733	.006	1.550	.014	1.440	.023	1.367	.032
1.857	.003	1.800	.003	1.600	.008	1.480	.015	1.400	.022
1.929	.002	1.867	.002	1.650	.005	1.520	.009	1.433	.015
2.000	.001	1.933	.001+	1.700	.003	1.560	.006	1.467	.010
2.071	.000	2.000	.001−	1.750	.002	1.600	.004	1.500	.006
		2.067	.000	1.800	.001	1.640	.002	1.533	.004
				1.850	.000	1.680	.001+	1.567	.002+
						1.720	.001−	1.600	.002−
						1.760	.000	1.633	.001+
								1.667	.001−
								1.700	.000

VARIETY, TYPE, YEAR, AND LOCATION EFFECTS ON THE CHEMICAL COMPOSITION OF PEANUTS

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In 1953 there were 1,538,000 acres of peanuts harvested in the United States from which a total yield of 787,125 tons was obtained *(6)*. Growing peanuts for the commercial production of vegetable fats, oils, and proteins and for human and animal consumption is becoming increasingly more important *(12)*. The kernel of the peanut *(Arachis hypogaea L.)* is one of the richest sources of vegetable oil and protein. Its oil content (45 to 50%) is approximately 10 times that of corn, and its protein (25 to 30%) about 2.5 times as much. In addition to being a rich source of these two important constituents the peanut also excels in its content of some of the nutritionally important B vitamins.

In attempts to improve the agronomic quality and yield of peanuts, breeding and selection studies were conducted for a number of years at the Tidewater Research Station at Holland, Virginia, by Batten *(3)*, and Beattie and Batten *(4)*. Workers at the Florida *(10, 16)* and Georgia Stations *(8, 9)* have also undertaken somewhat similar studies. Since the preponderance of breeding and selection work with peanuts has had as its chief objectives the improvement of quality and yield, there is consequently a dearth of reports in the literature on varietal differences in chemical composition. Perhaps the most complete project to be undertaken along this line was done at the Georgia Station and results of this work have been reported by Higgins, Holley, Pickett, and Wheeler *(9)*. In this report the authors state: "A cursory survey of more than 600 selected hybrid strains has shown considerable variation in oil and nitrogen content. A more detailed study of the chemical composition of 24 of these strains indicates considerable variation in other chemical characteristics and the possibility of selecting certain strains for special uses." Reporting on the analyses of groundnuts grown in India, Reddi and Giri *(13)* found a range of 40.9 to 50.2% fat in the kernel and 5.4 to 17.2 γ/g. thiamine in the defatted flour. These constituent differences were attributed to differences among the varieties.

Inasmuch as studies of varietal differences in the chemical composition of peanuts had apparently been somewhat neglected, a project was initiated in 1947 at the Tidewater Research Station at Holland, Virginia. In this study an attempt was made to obtain information on the composition of the kernels and hay of a number of standard peanut varieties and introductions. Year to year and location differences for the various constituents were also studied. This study, involving analyses of some 30 samples per variety and extending over a period of several years, offered an excellent opportunity to compare results thereby obtained with presently reported composition data on peanuts.

EXPERIMENTAL METHODS

Variety-year study. In order to obtain some preliminary information as to the relative chemical composition of some of the promising peanut varieties, 19 of these varieties were planted in 1947 in unreplicated, unrandomized field plots. Nuts from these plantings were analyzed for protein, ether extract, thiamine, and riboflavin. On the basis of data thereby obtained, and also by considering the agronomic quality of the nuts, 9 varieties and 3 introductions were selected for a subsequent statistically planned study. These varieties were planted in a randomized block design, using 6 field replications (50-ft. row) per variety. Five-year results were obtained for protein and ether extract, 4– for thiamine and riboflavin, 1– for niacin content of the kernels, and 2– for protein content of the hay. The varieties and their types used in this study are given in Table 1. The soil was prepared and fertilized and the peanuts were harvested, cured, and shelled in the manner customary to the peanut growing section of Virginia. Samples were prepared for analysis by grinding the kernels (including the skins) in a food chopper and the hay in a Wiley mill.

A. O. A. C. Methods of Analysis *(1)* were used for protein and ether extract determinations, thiamine and riboflavin were analyzed by methods used by the Southern Cooperative Group *(14)*, and niacin was determined microbiologically, as described in methods of Vitamin Assay *(2)*. Analysis of variance, as described by Snedecor *(15)*, was used for analyzing the data and Duncan's *(7)* Multiple Range Method was used for testing the significance thereof.

Variety-location study. In an attempt to obtain information on location as well as varietal effects on the chemical composition of peanuts, another study was undertaken in 1952. Protein, ether extract, thiamine, and riboflavin analyses of the kernels, and protein analyses of the hay were made on the 1952 crops of 10 varieties of Virginia-type peanuts grown at each of three locations (Table 3). The first 4 of these varieties had been recommended to the peanut growers of the Tidewater Section of Virginia by workers at the Research Station at Holland, and the last 6 were chosen because they were potentially promising varieties. Chemical composition data on these varieties were obviously of particular interest and importance. The 3 locations were chosen to represent 3 different soil types as follows: Holland (Onslow), Isle of Wight (Norfolk fine sandy loam) and Petersburg (heavy Norfolk fine sandy loam). Since all 3 locations were in Tidewater, Virginia, practically the same climatic conditions prevailed. The 10 varieties were planted at each of the 3 locations in a randomized block design using 4 field replications (50-ft. row) per variety. Soil preparation and fertilization, crop harvesting, curing, and shelling, were similar to those used for this area. Methods used for chemical and statistical analyses have been cited under the variety-year procedure.

RESULTS AND DISCUSSION

Variety-year study (Table 1). *Protein.* A range of 17.6%[a] indicates considerable varietal variation in the protein content of the kernels with variety 9 having the highest and 1 the lowest. The Spanish- was 2.6% higher in protein content than the Virginia-type. There was considerably more variation in protein content among the Spanish- than among the Virginia-type varieties as shown by mean square values of 104.9 and 2.6 respectively. Variety had 11 times[b] more influence on the protein content than did year.

A range of 55.2% indicates large varietal differences in the protein content of the hay with variety 3 having the highest; variety 1, though

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[a] See footnote 4, Table 1.

[b] This, and subsequent comparisons of factor effects, such as variety, location, etc., have been made by comparing the percentage range (footnote 4, Table 1) of a given constituent for a particular factor with the percentage range of the same constituent for the other factor under consideration.

TABLE 1

Variety, type, and year effects on the chemical composition (dry basis) of peanuts[1]

Variety name	Type	No. samples per variety	Kernels					Hay
			30	30	24	24	4[2]	12
		Variety No.	Protein[3] %	Ether extract[3] %	Thiamine[3] mg. %	Riboflavin[3] mg. %	Niacin[3] mg. %	Protein[3] %
153-342	Virginia	1	29.0[e]	46.9[b]	1.33[d]	0.157[b]	19.9[d, e, f]	12.8[b]
153-333	Virginia	2	29.4[f]	45.7[d, e]	1.31[d, e]	0.144[c]	26.3[a]	10.5[c, d, e, f]
149-637	Virginia	3	29.4[f]	44.5[f]	1.66[a]	0.241[a]	23.8[a, b, c]	14.9[a]
Va. bunch large	Virginia	4	29.4[f]	45.3[e]	1.25[e, f]	0.143[c, d]	25.7[a]	11.4[c]
Va. bunch small	Virginia	5	29.8[e]	45.5[d, e]	1.29[d, e]	0.139[d, e]	24.7[a, b]	10.4[c, d, e, f]
Va. runner	Virginia	6	29.8[e]	45.9[c, d, e]	1.19[f]	0.145[c]	24.4[a, b]	9.7[f]
Jumbo	Virginia	7	29.8[e]	44.5[f]	1.40[c]	0.145[c]	21.6[b, c, d]	10.5[c, d, e, f]
Spanish 2-B	Spanish	8	33.8[b]	46.1[c, d]	1.56[b]	0.130[f]	18.2[d, e, f]	9.6[f]
Valencia	Spanish	9	34.1[a]	45.7[d, e]	1.58[b]	0.126[f]	20.5[c, d, e]	10.0[e, f]
Light skin	Spanish	10	31.4[c]	48.7[a]	1.29[d, e]	0.153[b]	16.4[f]	11.0[c, d, e]
Ga. 207-3	Spanish	11	29.8[e]	48.7[a]	1.31[d, e]	0.141[c, d]	20.6[c, d, e]	11.1[c, d]
Dixie giant	Spanish	12	31.0[d]	46.5[b, c]	1.25[e, f]	0.137[c]	17.5[e, f]	10.1[d, e, f]
Mean			30.6	46.1	1.37	0.150	21.6	11.0
Percentage range[4]			17.6	9.4	39.5	91.3	60.4	55.2
FAO values (5)			27.0	45.7	0.89	0.127	16.9	11.1[5]
Virginia type mean			29.5	45.4	1.34	0.159	23.7	11.5
Spanish type mean			32.1	47.1	1.39	0.137	18.7	10.4
No. samples per year			72	72	72	72	48	72
1948			30.4[c]	45.5[c]	.....	.....	.....	.....
1949			30.5[b]	46.7[b]	1.37[c]	0.162[a]	.....	.....
1950			30.9[a]	45.1[d]	1.42[a]	0.159[b]	.....	.....
1951			30.6[b]	45.4[c]	1.40[b]	0.149[c]	.....	11.3
1952			30.4[c]	48.0[a]	1.30[d]	0.130[d]	21.6	10.8
Mean			30.6	46.1	1.37	0.150	21.6	11.0
Percentage range[4]			1.6	6.4	9.2	24.6	.....	4.6
F values:	Variety		276.1[**6]	37.1**	44.0**	408.5**	7.7**	37.5**
	Year		11.9**	66.2**	14.5**	285.9**	.....	10.5**
	V × Y		7.9**	3.2**	1.0	5.4**	.....	8.2**
	Type		1718.8**	161.8**	12.9**	636.3**	538.8**	52.0**
	% C.V.		5.3	4.8	8.1	5.3	19.1	6.1

[1] Six-replication/variety means.
[2] Four-replication/variety means.
[3] Data having superscript "a" are significantly different from those not having "a," those having "b" from those not having "b," etc. *(7)*.
[4] (Maximum value-Minimum value/Minimum value) × 100. Referred to in text as percentage range.
[5] Morrison's (11) value.
[6] ** Significant at 1% level.

TABLE 3

Variety and location effects on the chemical composition (dry basis) of Virginia-type peanuts[1]

Variety name	No. samples per variety	Kernels				Hay
		12	12	12	12	12
	Variety No.	Protein[2] %	Ether extract[2] %	Thiamine[2] mg. %	Riboflavin[2] mg. %	Protein[2] %
Va. Bunch Small	1	28.6[b]	48.2[a, b]	1.23[c, d]	0.132[d, e]	10.4[e]
Va. Bunch 46-2	2	28.3[b]	47.9[a, b]	1.19[e]	0.130[e]	11.4[a, b]
Holland Station Runner	3	28.7[b]	47.9[a, b]	1.14[e]	0.140[b, c]	10.7[c, d, e]
Check—Farmer's Stock	4	28.8[b]	47.7[b, c]	1.24[c, d]	0.139[b, c]	10.9[b, c, d, e]
Va. Bunch 46-1	5	28.6[b]	48.5[a]	1.16[e]	0.139[b, c]	11.2[a, b, c, d]
J-11-L	6	30.0[a]	46.9[c]	1.35[a]	0.147[a, b]	10.6[d, e]
119-24	7	29.1[b]	47.0[c]	1.28[b, c]	0.153[a]	10.8[b, c, d, e]
124-681	8	29.0[b]	48.2[a, b]	1.32[a, b]	0.138[d]	11.0[a, b, c, d, e]
149-645	9	28.6[b]	47.5[b, c]	1.25[c, d]	0.145[b, c]	11.5[a]
153-333	10	28.9[b]	47.5[b, c]	1.32[a, b]	0.145[b, c]	11.3[a, b, c]
Mean		28.9	47.5	1.25	0.141	11.0
Percentage range[3]		6.0	3.4	18.4	17.7	10.6
FAO values (5)		27.0	45.7	0.89	0.127	11.1[4]
No. samples per location		40	40	40	40	40
Holland		29.8[a]	48.1[a]	1.26[b]	0.136[b]	10.3[b]
Isle of Wight		28.7[b]	47.5[b]	1.10[c]	0.128[c]	10.3[b]
Petersburg		28.1[c]	47.6[b]	1.38[a]	0.158[a]	12.3[a]
Mean		28.9	47.7	1.25	0.141	11.0
Percentage range[3]		6.0	1.3	25.5	23.4	22.3
F values: Variety		3.5**[5]	4.4**	11.3**	7.4**	3.4**
Location		40.0**	5.1**	153.4**	130.8**	110.5**
V × L		1.3	0.9	1.5	2.4**	1.6
% C.V.		3.0	1.8	5.8	4.7	6.5

[1] Four-replication/variety means.
[2] See footnote 3, Table 1, for explanation of superscript letters.
[3] See footnote 4, Table 1.
[4] Morrison's (11) value.
[5] ** Significant at 1% level.

lower than 3, having considerably more than the others; and variety 6 and 8 having the lowest. It is interesting to note that the protein content of variety 3 hay was the highest while its kernel protein was among the lowest. The protein content of the hay of the Virginia-type was 1.1% more than that of the Spanish-, whereas the reverse was true for the kernels. Mean square values of 39.5 and 3.7 indicate greater varietal variation in hay protein content among the Virginia than among the Spanish type, respectively. This was also the reverse of that obtained for the kernels. The variety effect on the protein content of the hay was 12 times the influence due to year.

Ether extract. A 9.4% range, though not as large as for protein, indicated considerable varietal differences in the ether extract content of the kernels with varieties 10 and 11 having the highest and 3 and 7 the lowest. The small difference in ether extract content between the Virginia and Spanish types was negligible. Mean square values of 279.5 and 628.7 indicates that there were greater varietal variations among the Spanish than among the Virginia type. Variety effect was only 1.5 times the year influence for ether extract content.

Thiamine. A 39.5% range in thiamine content of the kernels indicates large varietal variations with variety 3 having the highest and 6 the lowest. The 0.05 mg.% difference in thiamine content of the kernels of the two types was negligible. The small mean square values of 0.62 and 0.56 show that there were very little varietal differences in thiamine content within each type. Variety affected the thiamine content of the kernels 4.3 times as much as year. The 9.2% yearly range, however, was significant and was large enough to be considered important.

Riboflavin. The 91.3% range in riboflavin content of the kernels represents the largest variation among the varieties of any of the constituents studied with variety 3 having the highest and 8 the lowest. It should be noted, however, that because of its unusually high riboflavin content, variety 3 was the largest contributing factor to the wide range of this constituent. Both variety and year effects on the riboflavin content of the kernels were important with variety having 3.7 times as much influence as year. The 0.022 mg.% difference in riboflavin content of the kernels of the two types was negligible. Variations among the varieties within each type were small as indicated by mean square values of 0.033 and 0.026 for the Virginia and the Spanish type, respectively.

Niacin. A 60.4% range in niacin content of the kernels indicates large varietal variations with variety 2 having the highest and variety 10 the lowest. The Virginia type had 5.0 mg.% more niacin than the Spanish type of kernels. A higher varietal variation in the niacin content of the kernels occurred among the Virginia than among the Spanish type as indicated by mean square values of 61.4 and 41.7, respectively; the latter value, however, was not significant.

Factors causing changes in content of the foregoing constituents which were large enough to be considered of practical importance are as follows: Variety effect on the protein, ether extract, and niacin content of the kernels and protein content of the hay; variety and year effect on the thiamine and riboflavin content of the kernels; type (Virginia or Spanish)

effect on the protein and niacin content of the kernels and protein content of the hay; and effects of varietal variations within types on the protein and ether extract content of the kernels and the protein content of the hay.

Peanut kernels of the Variety-year study (Table 1) contained 13, 1, 54, 18 and 28% more protein, ether extract, thiamine, riboflavin, and niacin, respectively, than the values reported in the F. A. O. Tables *(5)*. The 11.0% protein in the hay was practically the same as Morrison's *(11)* value of 11.1%.

Highly significant variety × year interactions indicated that varieties ranked differently among themselves from year to year for all the constituents except thiamine. Attention should be called to the fact that coefficients of variation, expressed as per cent (% c.v.), for the different constituents were, with the exception of niacin, well below the accepted figure for this type of experiment. The 19.1% c.v. for niacin is somewhat larger than that usually allowed for microbiological assay methods. Because of this high % c.v. and also because this constituent was determined on only one crop of nuts, results for niacin should not be considered as reliable as those for the other constituents.

It will be noted that variety 3 was much higher than any of the others in thiamine and riboflavin content of the kernels, protein in the hay, and the second highest in niacin. Practical observations have indicated that this variety is inherently low in agronomic quality and yield. In spite of this fact it is recommended that, because of its high nutrient content, it be used by plant breeders for crossing with high quality, high yielding varieties as a possible means of increasing the vitamins in the kernels and protein in the hay, at the same time retaining the high agronomic quality and yield of the varieties with which it is crossed.

Correlation coefficients (Table 2). In order to obtain information on the relationship of the different constituents to each other, correlation coefficients (r) were calculated for the various nutrients by use of the method described by Snedecor *(15)*. These coefficients have been designated by numbers which will be referred to in the following discussion. With the exception of the significantly positive correlation coefficient for No. 2, all the other significant r values were negative, thereby indicating an

TABLE 2

Correlation coefficients (r) of the chemical constituents of peanuts

Coefficient No.	Constituent comparisons	D.F.	r	Significance[1]
1	Protein: Ether extract	59	+0.071	N.S.
2	Protein: Thiamine	47	+0.345	*
3	Protein: Riboflavin	47	−0.371	**
4	Protein: Niacin	47	−0.372	**
5	Ether extract: Thiamine	47	−0.394	**
6	Ether extract: Riboflavin	47	−0.412	**
7	Ether extract: Niacin	47	−0.301	*
8	Thiamine: Riboflavin	47	−0.425	**
9	Thiamine: Niacin	47	−0.014	N.S.
10	Riboflavin: Niacin	47	−0.028	N.S.
11	Protein in kernels: protein in hay	143	−0.287	**

[1] * and ** Significant at 5% and 1% levels.
N.S.—Not significant.

inverse relationship between these constituents. No. 8 had the highest and No. 7 the lowest significant correlations while those for Nos. 1, 9, and 10 were not significant. Though there were 6 highly significant and 2 significant correlations, they should all be taken with reservation due to the fact that r values below 0.7 or 0.8 are considered, by most workers, as having little real significance.

Variety-location study (Table 3). *Protein.* Variety 6 had the highest protein content of the kernels and was the only one which was significantly different from the others. Due to the fact that both varietal and location ranges were only 6%, and that only one variety was significantly different from the others, the effects of these two factors on the protein content of the kernels were of little importance. Location effect on hay protein was approximately twice that due to variety as indicated by 22.3 and 10.6% ranges, respectively.

Ether extract. Variety 5 had the highest ether extract content of the kernels and 6 had the lowest. Both variety and location had very little effect on the ether extract content of the peanuts as shown by ranges of only 3.4 and 1.3% respectively.

Thiamine. Variety 6 had the highest and 3 the lowest thiamine content of the kernels. The Petersburg kernels had the highest and the Isle of Wight the lowest thiamine content. Both variety and location affected the thiamine content of the kernels with location having 38.6% more influence than variety.

Riboflavin. Variety 7 had the highest and 2 the lowest riboflavin content. The Petersburg kernels had the highest and Isle of Wight the lowest riboflavin content. As with thiamine, both variety and location affected the riboflavin content of the kernels with locations having 32.2% more influence than varieties.

Variety and location effects on the protein content of the hay and on the thiamine, and riboflavin contents of the kernels were large enough to be considered of practical importance. The kernels of the Variety-location study (Table 3) contained 7, 4, 40, and 11% more protein, ether extract, thiamine, and riboflavin than values reported in F. A. O. Tables *(5)*. The 11.0% hay protein was practically the same as Morrison's *(11)* value of 11.1%.

A highly significant variety × location interaction was obtained only for riboflavin, thereby indicating that the varieties ranked the same among themselves at each of the 3 locations for all the constituents except riboflavin. It will also be noted that the coefficients of variability, expressed as per cent (% c.v.), for all the constituents were well below the accepted figure for this type of experiment. Though no practical recommendations are attempted at this time, because of lack of yield data, it should be pointed out that variety 6 was, in general, superior to the other varieties in its chemical composition and that Petersburg was the location at which optimum chemical composition was attained. It is suggested that further work be undertaken on variety 6 and/or its crosses as a possible means of developing varieties which are superior in quality, yield, and nutritive value and which can be recommended to the peanut grower.

SUMMARY AND CONCLUSIONS

The effects of variety, type, and year on their chemical composition were studied on 7 varieties of the Virginia and 5 varieties of the Spanish type of peanuts, on which 5-year protein and ether extract, 4-year thiamine and riboflavin, and 1-year niacin analyses of the kernels and 2-year protein analyses of the hay were made. The effects of variety, and location on their chemical composition were studied on 10 varieties of the Virginia type of peanuts of the 1952 crops from 3 different locations in Tidewater, Virginia. Protein, ether extract, thiamine, and riboflavin analyses of the kernels, and protein analyses of the hay were made. The following pertinent results were obtained:

a. In the Variety-year study, variety had a greater influence on the contents of all the constituents in peanuts than did year but year also had an important effect on thiamine, and riboflavin.

b. In the Variety-location study, location had a greater influence on thiamine, riboflavin, and hay protein content than variety while neither factor had any influence of importance on protein or ether extract.

c. Spanish type peanuts were higher in protein, while the Virginia type was higher in niacin content of the kernels, and protein in the hay.

d. Effects of varietal variation within types on the protein and ether extract contents of the kernel and the protein content of the hay were important. The higher the content of a particular constituent in peanuts the greater its varietal variability.

e. The variety × year interaction was significant for all constituents studied except thiamine. The variety × location interaction was significant only for riboflavin.

f. Mean protein, riboflavin, and niacin contents of the kernels were considerably higher, and the mean thiamine content 50% higher than F. A. O. *(5)* values. Protein content of the hay was practically the same as Morrison's *(11)* value.

g. Significant r values were obtained in 8 out of 11 different composition data comparisons but were found to be too low to be of practical importance.

h. Variety J-11-L and Introduction 149-637, though not high in agronomic quality and yield, were highest in nutritive value and could well be used in future peanut breeding studies as a possible means of increasing the nutritive value of high yielding varieties.

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PHENOLIC COMPOUNDS IN POTATO TISSUE*

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It has long been known that the darkening of cooked potatoes involves an oxidation to produce the blue-gray-black color especially prominent at the stem-end portion of a tuber. It has been suggested that the colorless precursor was produced by tyrosinase (phenolase) action *(1, 8, 9)*. Tyrosine or other free alpha amino acids, catechols or dihydroxy-phenylalanine have been listed as possible substrates *(1, 4, 7)*. More recently, Juul *(4)* reported that ortho diphenols (probably caffeic acid) were involved in the stem-end darkening of potatoes after cooking, and Johnson and Schaal *(3)* have identified tyrosine and chlorogenic acid in potato tissue.

The widespread occurrence of darkening of cooked potatoes grown in the northern regions of the United States and of Europe warrants further studies of the components involved in this discoloration. The plan of study was to compare the total tannin (tannin and tyrosine) content of various portions of potato tubers and of a number of varieties and to correlate such information with the degree of darkening. Paper chromatographic and spectro-photometric identification of some phenolic compounds were made.

EXPERIMENTAL PROCEDURE

The samples for total tannin content included a group of Wisconsin market Chippewa, Russet Burbank, and Sebago potatoes which had been stored from 4 to 6 months. Market samples of Russet Burbanks grown in Idaho and Triumphs grown in Florida were also used. The potatoes were stored at a temperature of 38° to 42°F. and held at room temperature for one week prior to the tests.

A judging panel of 6 or 7 trained judges scored the degree of darkening of a quarter of each potato, using a scale of 1 to 10, with the score of 10 for perfect white or cream color and 7 for an acceptable color. These quarters of potatoes had been boiled approximately 30 minutes to an internal temperature of 98-100°C., skinned, and exposed to the air for one hour to permit maximum color development before scoring. The cooking was done in tap water of 20 degrees hardness and in similar aluminum saucepans, as is common household procedure.

Other portions of the same tubers were extracted in a Waring blender for 5 minutes according to the method of Johnson and Schaal *(3)*, using 95% ethyl alcohol in the amount of 3 ml. to 1 g. of potato. The extract was filtered and 1 ml. was removed for the tannin determination. The rest of the extract was concentrated under reduced pressure to dryness and in order to remove any fatty or waxy material, the resulting powder was extracted three times with 5 ml. petroleum ether in a 15 ml. centrifuge tube, centrifuged and the petroleum ether decanted each time. The dry powder was dissolved with distilled water (1 ml. per 100 g. equivalent potato tissue) and then stored in a refrigerator until used for the paper chromatography. The same procedures were used for the potato peeling, except that 0.3 ml. of extract was removed for the total tannin determinations.

On some other tubers, comparison was made of the amount of tannin present in the areas which tended to darken more with the amount present in the lighter flesh of

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the same potatoes. Twenty tubers of the Chippewa I potatoes, which had been shown to darken markedly, were used for this comparison. The tubers were cut into halves longitudinally and half of each tuber was boiled for 30 minutes and exposed to the air. By comparison with the color plates of Maerz and Paul *(5)* these tissues were divided into three groups: dark gray, light gray and white or cream color. The tissues from the raw half of each tuber corresponding to the various colored areas were, when present, cut out, combined and extracted as above.

The amount of tannins in the potato extracts was determined as tannic acid using the method of Rosenblatt and Peluso *(6)*. Since this method is generally used in determining phenolic materials, the L-tyrosine as well as tannins of the different potato tissues was included in the quantities recorded under the term, *total tannin content*.

The tannin preparations were subjected to characterization and identification by application of paper chromatography and ultraviolet absorption techniques. The paper chromatographic method developed by Williams and Kirby *(10)* based on one-dimensional capillary ascent was followed. The paper chromatography was carried out on Whatman No. 1 filter paper strips using n-butanol-acetic acid-water (5:1:4) as developing solvent. The samples applied to the paper were not measured by means of micropipettes. However, handmade pipettes of marked capillary tubes were used, so comparative size samples were always employed. The paper chromatograph was allowed to develop from 6 to 8 hours. Then it was removed, allowed to dry, and sprayed with modified Folin-Denis reagent (1 part of reagent, 1 part of distilled water, and 2 parts of 95% ethyl alcohol) and exposed to the fumes from concentrated NH_4OH. The R_f values of the deep blue color were measured to the leading edge of each spot instead of the center of the spot, as this gave more consistent results. Paper chromatography of some pure phenolic substances, such as chlorogenic acid, D-catechin, pyrocatechin, caffeic acid and L-tyrosine were run at the same time for comparative purposes.

Ultraviolet absorption determinations were made on a Beckman Model DU Quartz Spectrophotometer of chlorogenic acid, caffeic acid, dihydroxy phenylalanine, d-catechol and tyrosine and of the extracts of the dark tissue, the peelings and the center section of potatoes labeled Wisconsin Chippewas I.

RESULTS AND DISCUSSION

The data presented in Table 1 show that tannins (tannin and tyrosine) were much more concentrated in the skins than in the center flesh of potatoes. There was slightly less tannin in the bud end than in the stem end. Among the 7 lots of potatoes tested, Chippewas I had the highest tannin content both in the skins and in the center flesh. On the other hand, Chippewas II had a lower tanin content than the other potatoes tested. The lot of Idaho Russet Burbanks was higher in tannin content in both skins and flesh than the Wisconsin Russet Burbanks tested.

A study of a scattergram of color scores of individual samples plotted against the tannin content of the stem-end portions of those tubers failed to show a definite trend for all samples. If the samples of Chippewa II were omitted, the relation appears to be a higher color score with a lower tannin content. If, however, Chippewa II was included and not Chippewa I, the opposite relationship would appear to predominate. Thus, no conclusion was possible.

Another approach to the tannin-color problem was the comparison of sections of the same tubers which noticeably differed in grayness. These were found to differ in tannin content. The lighter flesh from 12 tubers gave an average of 0.63 mg./g., the light grayish flesh from 7 tubers gave an average of 0.74 mg./g. and the dark gray flesh from 20 tubers gave an average of 0.98 mg./g. Thus, there appeared to be a degradation of tannin content along with the gradation of discoloration.

TABLE 1

The tannin content (mg/g) and color scores of 7 lots of potatoes (9 replicates each)

Lot	From skin of bud-end		From skin of stem-end		Center flesh		Color scores	
	Range	Average	Range	Average	Range	Average	Range	Average
Wisconsin Chippewa I	1.84-2.67	2.27	1.77-2.52	2.12	0.70-0.88	0.79	1.2-3.7	2.5
Wisconsin Chippewa II	1.06-1.37	1.20	0.96-1.23	1.13	0.42-0.65	0.55	3.1-4.6	4.1
Wisconsin Russet Burbank I	1.72-2.07	1.90	1.51-1.81	1.67	0.64-0.78	0.69	4.2-5.7	4.9
Wisconsin Russet Burbank II	1.47-1.97	1.77	1.50-1.82	1.68	0.68-0.82	0.75	5.2-6.6	5.8
Wisconsin Sebago	1.58-2.11	1.83	1.58-2.10	1.79	0.69-0.81	0.74	4.1-6.1	5.5
Idaho Russet Burbank	1.86-2.55	2.15	1.70-2.25	1.98	0.42-0.61	0.54	5.9-7.6	6.9
Florida Triumph	1.49-1.83	1.65	1.44-1.79	1.65	0.53-0.86	0.73	6.6-8.1	7.2

The concentrated extracts from the skin and the center flesh of the 7 lots of potatoes were studied by the paper chromatographic method. Figure 1 shows typical papergrams prepared by spraying with modified Folin-Denis reagent and treating with ammonia fumes to alkalize the reagent. Nos. 1, 2 and 3 are the papergrams of L-tyrosine, chlorogenic acid and caffeic acid, respectively. Nos. 4, 5 and 6 show the papergrams of Chippewas I skin, the discolored flesh and the lighter center flesh, respectively. The papergrams of the skin and the center flesh extract from the other 6 lots of potatoes had spots with similar R_f values, which are summarized as follows:

Substances	*R_f values*	*Identified component*
l-tyrosine	0.20-0.25	
Chlorogenic acid	0.67-0.70	
d-catechin	0.77-0.80	
Caffeic acid	0.90-0.93	
Pyrocatechin	0.96-0.98	
Tannic acid	0.97-0.99	
Skin extracts:		
Spot 1	0.65-0.70	Chlorogenic acid
Spot 2	0.91-0.95	Caffeic acid
Discolored flesh extract:		
Spot 1	0.18-0.23	l-tyrosine
Spot 2	0.65-0.70	Chlorogenic acid
Center flesh extract:		
Spot 1	0.19-0.25	l-tyrosine
Spot 2	0.48-0.55	?
Spot 3	0.68-0.70	Chlorogenic acid

Figure 1. Papergrams of L-tyrosine, chlorogenic acid and of extracts of the skin, discolored flesh and center light-colored flesh of Chippewa I potatoes.

Papergram No. 4, from the Chippewa skin extract, shows high chlorogenic acid and some caffeic acid but very low L-tyrosine content. The dark flesh extract, No. 5, reveals the presence of chlorogenic acid and L-tyrosine. The center flesh extract, No. 6, reveals small amount of L-tyrosine, a weak test for chlorogenic acid and another component unidentified.

Figure 2 shows the ultraviolet absorption spectra of L-tyrosine, caffeic acid and chlorogenic acid. Figure 3 shows the ultraviolet absorption spectra of the extracts of the skin, the dark flesh and the center flesh. Spectrum No. 1 of the skin extract reveals the presence of chlorogenic acid and another caffetannin probably caffeic acid. Spectrum No. 2 of the dark flesh extract has slight bands at 268 mμ and 324 mμ which indicate L-tyrosine and chlorogenic acid. Spectrum No. 3, the center flesh extract, has a strong band at 268 mμ which is due to L-tyrosine and a very slight band at 324 mμ indicates traces of chlorogenic acid. Thus, the absorption curves confirmed the presence of the same substances in the potato tissues as had been shown by the chromatographic technique. The absorption spectra of dihydroxyphenylalanine (DOPA) and of D-catechol, were also determined. In each there were maxima at 280 mμ and minima at 250 mμ. Such bands were not noted in the absorption curves of the potato tissues.

Thus, chlorogenic acid was present in the skins, and to some extent in discolored areas as well as the center flesh of the potatoes whereas L-tyrosine was present in detectable amounts only in the discolored areas and the lighter center flesh of the potatoes. In the skin extracts, a second spot which showed on the papergrams was identified as caffeic acid. It is of interest to compare these findings with those of other workers. Claggett

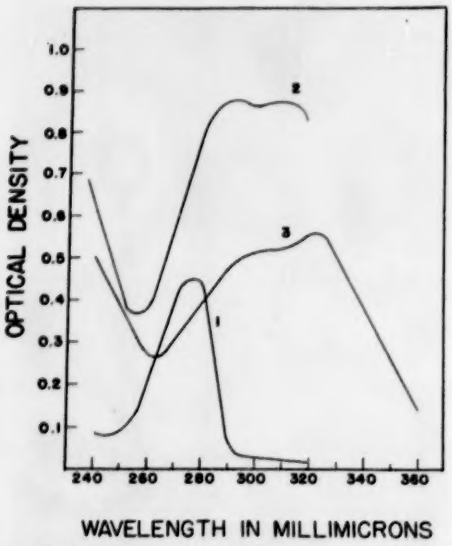

Figure 2. Ultra violet absorption spectra of tyrosine (curve 1), caffeic acid (2) and chlorogenic acid (3).

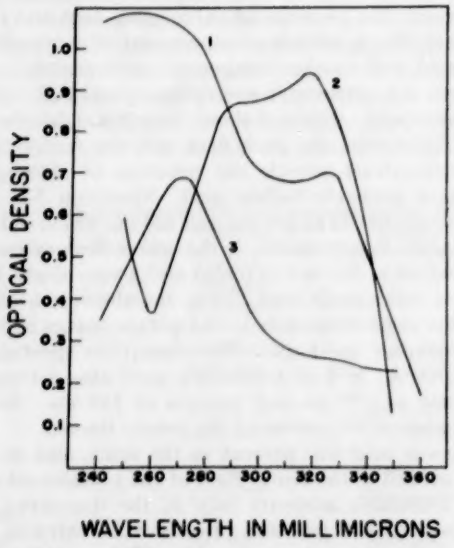

Figure 3. Ultra violet absorption spectra of extracts from the dark flesh (curve 1), the skin (2) and of the light center flesh of Chippewa I potatoes.

and Tottingham *(1)* considered DOPA to be present, Johnson and Schaal *(3)* reported the presence of chlorogenic acid and L-tyrosine in significant quantities in potatoes and Juul *(4)* suggested caffeic acid. Johnson *(2)* found by the paper chromatographic method much larger amount of chlorogenic acid than DOPA present in potatoes, and since chlorogenic acid is a natural substrate for tyrosinase, he suggested that chlorogenic acid rather than DOPA was involved in the terminal oxidase system of white potatoes. In the dark areas or in darkening tubers, abnormal conditions may involve the oxidase system and its substrates in a manner not as yet clear.

SUMMARY

Tannins were found to be more concentrated in the skins than in the center flesh of potatoes. There was slightly greater content of tannins in the stem end than the bud end. No correlation could be established between color scores and tannin content of the various varieties and samples. However, tannins were found to be more concentrated in the dark grayish flesh than in the lighter flesh. Whether a relationship might exist between the content of tannin-like substances and the regions which developed dark pigments after cooking and exposure to air must be left to research on the specific components involved.

By the paper chromatographic technique, chlorogenic acid was found to be the only tannin-like substance present in detectable amounts in extracts obtained from all three of the types of potato tissues, the skins, the discolored areas, and the center flesh. In addition, in the skin extract, caffeic acid was present; L-tyrosine was found to be present in detectable amounts in the discolored areas and the center flesh of the tubers. The presence of these substances was confirmed in all cases by absorption curve methods.

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DEVELOPMENT OF A SCALE FOR MEASURING SOLDIERS' FOOD PREFERENCES[a, b]

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Acceptability, always an important consideration in food development and utilization, is particularly so in problems of mass feeding such as those encountered in designing rations for the Armed Forces. Military rations must be adjusted to the preferences of the entire population of Service men. Even foods that are extremely well-liked, but by only a small proportion of the consumers, are unsuited for military use. Items must be selected which have satisfactory average preference and are disliked by as small a proportion of the population as possible. For efficient selection, methods are required which will determine acceptability in different kinds of situations, including laboratory and field pretests of actual food items, and which will serve for investigations of food preferences. All such tests depend on the use of psychological measurement to reduce to a common scale the subjective attitudes of many people. Experience has shown that the approach commonly called the rating scale method, or, more completely, the method of successive intervals, is the most appropriate and efficient.

In 1949 a device known as the "hedonic scale" was developed at the Quartermaster Food and Container Institute for the Armed Forces and has become the standard instrument for use by the QM Corps in laboratory and field tests of acceptability *(6)*. Although it has provided usable information about food preference, certain deficiencies in the scale were noted. Since accurate measurement of food preferences is vital in food research, it became important not merely to correct recognized defects, but to establish with a reasonable degree of certainty a method which would be optimal for military use. In 1951 the Psychometric Laboratory at the University of Chicago undertook such a project.

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[a] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, Chicago, Ill., and has been assigned number 524 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

[b] Presented at the Fourteenth Annual Meeting of the Institute of Food Technologists, Los Angeles, California, June 29, 1954.

Problem. Inspection of a rating scale may suggest that widths of the intervals should be equal. However, there is never assurance that any one interval is of the same width, psychologically, as any other. In fact, typically, there is evidence of gross inequality. The reasonable objective is a rating scale for which no one would question that the successive intervals are in the proper ordinal position, and where all subjects understand and use the intervals in about the same way. When that has been achieved, the variance in the ratings of a particular food may be interpreted as indicating different levels of preference for that food, rather than different ways of understanding the rating scale.

The choice of words or phrases to label the scale intervals is of first importance, since these verbal anchors serve both to convey the idea of the successive order of the intervals and to make clear to the respondents the meaning of the response continuum. The value of a scale will be reduced to the extent to which the words and phrases are ambiguous, or are not definitely in an order of meaning corresponding with the physical order of the scale intervals. Scales may vary in other ways, too. Among the most important are (a) the number of intervals, ((b) whether or not the scale is balanced, i.e., has an equal number of positive and negative intervals, and (c) whether or not a "neutral" category is included. All of these variables are included in the present study.

PROCEDURE AND RESULTS

The research reported here involves a number of interrelated phases. The first task was to develop and evaluate a potential "food preference vocabulary." Following this, two series of scales, each of which embodies certain hypotheses regarding the other important variables, were designed and evaluated in field surveys. Pertinent procedural details are included in the discussion of results.

Selection of descriptive phrases. Fifty-one words and phrases were selected for investigation. Part of this list resulted from a pilot study with a group of Army men; other elements were included because of their frequent use in preference questionnaires or their apparent logical suitability. Subjects were approximately 900 soldiers from Fort Lee, Virginia, selected on the basis of educational background to be representative of Army enlisted men. Figure 1 shows the rating scale and examples of items that were included. The subjects were told: "The items are words and phrases that people use to show like or dislike for foods. For each item make a check mark in the box which best shows what the word or phrase means to you."

The methods of analysis used in this phase of study have been described elsewhere *(4)*. Briefly, the analysis provides for determination of a psychological continuum of meaning which exhibits the characteristics of an equal interval scale, the method being based on the assumption that each phrase has a modal meaning about which the various meanings attributed to it by the respondents are normally distributed. A scale value and standard deviation are derived graphically for each item. The former may be considered the "average meaning" for the phrase, and the latter a measure of its relative ambiguity. In Table 1 appear these indices for all of the phrases. It will be noted that size of the standard deviation is not independent of scale value, for as scale values depart from zero, standard deviation values tend to increase. This result correctly is interpreted as indicating relatively greater ambiguity of meaning of "extreme" phrases than of "neutral" phrases. That this is a reasonable finding is clear; as the meaning of a phrase departs from that of neutrality, it becomes more likely that individuals will exhibit greater disagreement as to the precise position of the phrase on the meaning continuum.

An important aspect of the distributions, which is not apparent from the numerical data alone, is shown by graphical plots (on binomial probability paper) of cumulative proportions of responses against the scale values of the boundaries of the successive

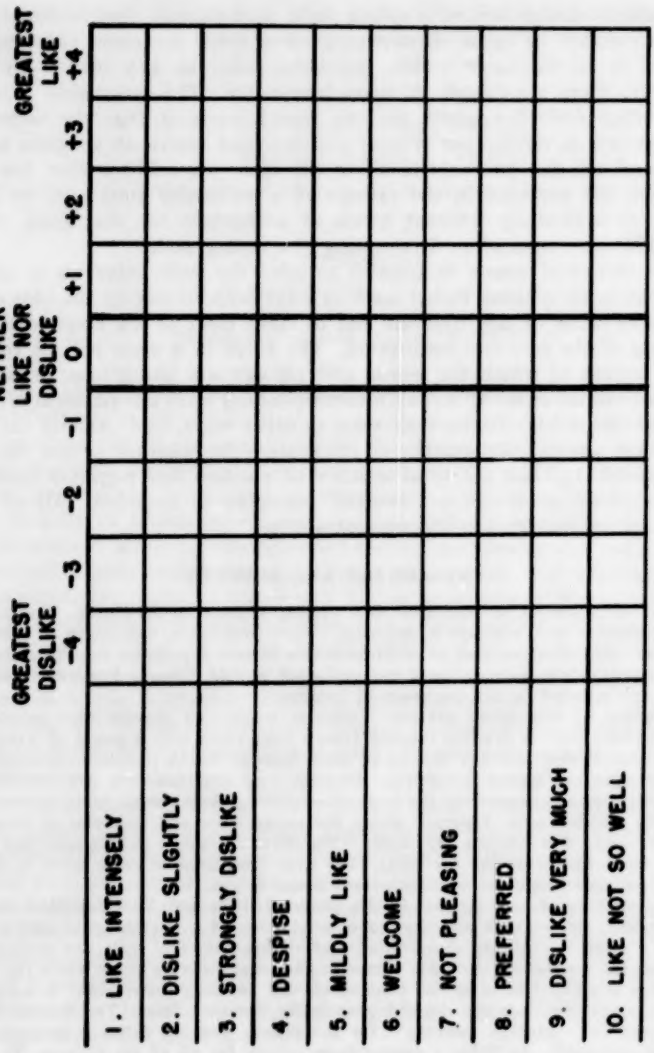

Figure 1. Scale and examples of phrases used in the meaning study.

intervals. Departures from linearity on these graphs illustrate failures in the assumption of normality. Graphs for three of the phrases are shown in Figure 2. Included are two of the six phrases which show marked departure from normality, together with one phrase, "preferred," for which departure is slight. "Dislike moderately" illustrates a positively skewed distribution. A significant number of men marked it on the "like" side of neutral. "Average" exhibits a bimodal distribution; one group of men marked it at the center of the scale and another group placed it two steps above the center. Each instance of a non-normal distribution can be diagnosed as an indica-

TABLE 1
Scale values and standard deviations for 51 descriptive phrases included in the word meaning study

Phrase	Scale value	Standard deviation
Best of all	6.15	2.48
Favorite	4.68	2.18
Like extremely	4.16	1.62
Like intensely	4.05	1.59
Excellent	3.71	1.01
Wonderful	3.51	.97
Strongly like	2.96	.69
Like very much	2.91	.60
Mighty fine	2.88	.67
Especially good	2.86	.82
Highly favorable	2.81	.66
Like very well	2.60	.78
Very good	2.56	.87
Like quite a bit	2.32	.52
Enjoy	2.21	.86
Preferred	1.98	1.17
Good	1.91	.76
Welcome	1.77	1.18
Tasty	1.76	.92
Pleasing	1.58	.65
Like fairly well	1.51	.59
Like	1.35	.77
Like moderately	1.12	.61
OK	.87	1.24
Average	.86	1.08
Mildly like	.85	.47
Fair	.78	.85
Acceptable	.73	.66
Only fair	.71	.64
Like slightly	.69	.32
Neutral	.02	.18
Like not so well	−.30	1.07
Like not so much	−.41	.94
Dislike slightly	−.59	.27
Mildly dislike	−.74	.35
Not pleasing	−.83	.67
Don't care for it	−1.10	.84
Dislike moderately	−1.20	.41
Poor	−1.55	.87
Dislike	−1.58	.94
Don't like	−1.81	.97
Bad	−2.02	.80
Highly unfavorable	−2.16	1.37
Strongly dislike	−2.37	.53
Dislike very much	−2.49	.64
Very bad	−2.53	.64
Terrible	−3.09	.98
Dislike intensely	−3.33	1.39
Loathe	−3.76	3.54
Dislike extremely	−4.32	1.86
Despise	−6.44	3.62

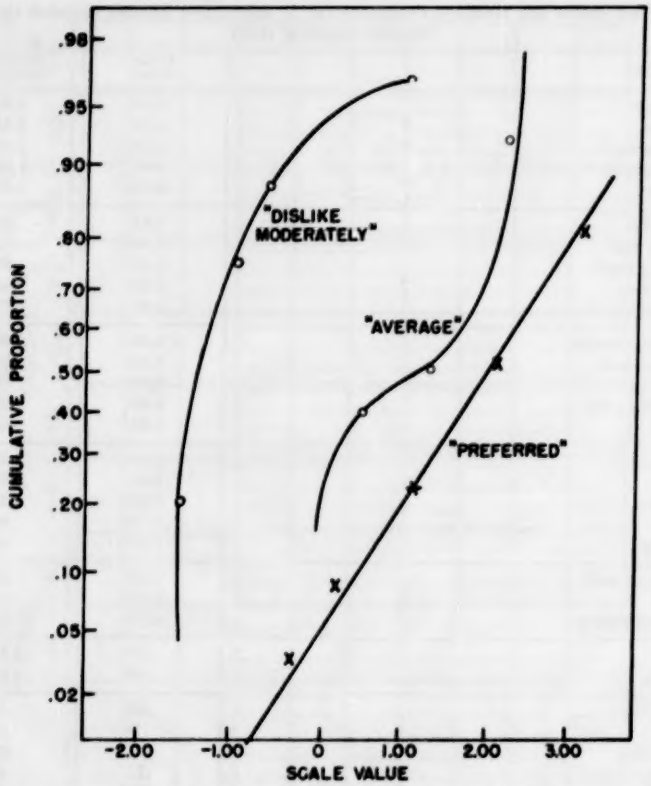

Figure 2. Graphical plots for three descriptive phrases displaying different types of distribution.

tion of some particular confusion regarding the meaning of the phrase.

On the basis of these findings, descriptive phrases could be selected for use in preference scales on the basis of their known "average meanings," low ambiguity, and slight departures from normality.

Comparison of scales. The nine different scale types shown in Figure 3 were investigated. Note that they vary in length and that various combinations of phrases are employed to describe the intervals. The middle interval is eliminated in Nos. 4, 7, and 9. Nos. 8 and 9 are "unbalanced," with fewer "dislike" than "like" categories. No. 1 is the hedonic scale currently used by the QM Corps. Scales 1-5 were included in the first field test, conducted in June 1952. The respondents were 3600 enlisted men sampled from the four Army posts on the eastern seaboard. The second field test was administered at Fort Bragg, N. C. in August 1953 to 1800 men, and included scales 1 and 6-9.

Each test was ostensibly a preference survey of 20 food items which had been selected, on the basis of previous survey results, to cover a wide range of preference. All 9 questionnaires studied include the same food items, and differ only in regard to the rating scales. Respondents were simply instructed to check the reply which best showed how much they liked or disliked each food. Questionnaires were administered in class sessions, each of which included no more than 100 men, and the five scale types were systematically and evenly distributed in each group.

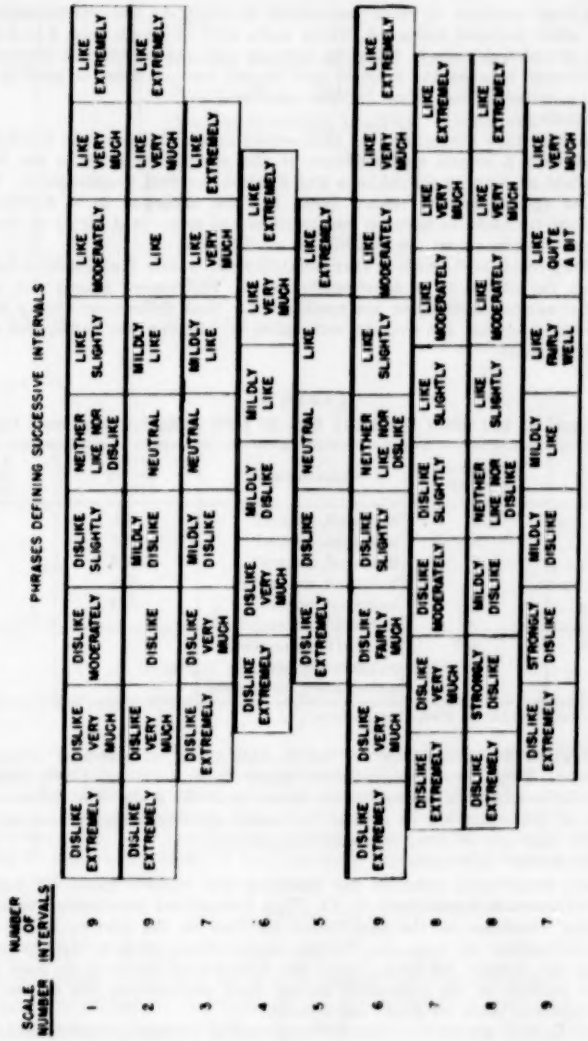
SCALE NUMBER
NUMBER OF INTERVALS
PHRASES DEFINING SUCCESSIVE INTERVALS
1 9 DISLIKE EXTREMELY | DISLIKE VERY MUCH | DISLIKE MODERATELY | DISLIKE SLIGHTLY | NEITHER LIKE NOR DISLIKE | LIKE SLIGHTLY | LIKE MODERATELY | LIKE VERY MUCH | LIKE EXTREMELY
2 9 DISLIKE EXTREMELY | DISLIKE VERY MUCH | DISLIKE | MILDLY DISLIKE | NEUTRAL | MILDLY LIKE | LIKE | LIKE VERY MUCH | LIKE EXTREMELY
3 7 DISLIKE EXTREMELY | DISLIKE VERY MUCH | MILDLY DISLIKE | NEUTRAL | MILDLY LIKE | LIKE VERY MUCH | LIKE EXTREMELY
4 6 DISLIKE EXTREMELY | DISLIKE VERY MUCH | MILDLY DISLIKE | MILDLY LIKE | LIKE VERY MUCH | LIKE EXTREMELY
5 5 DISLIKE EXTREMELY | DISLIKE | NEUTRAL | LIKE | LIKE EXTREMELY
6 9 DISLIKE EXTREMELY | DISLIKE VERY MUCH | DISLIKE FAIRLY MUCH | DISLIKE SLIGHTLY | NEITHER LIKE NOR DISLIKE | LIKE SLIGHTLY | LIKE MODERATELY | LIKE VERY MUCH | LIKE EXTREMELY
7 8 DISLIKE EXTREMELY | DISLIKE VERY MUCH | DISLIKE MODERATELY | DISLIKE SLIGHTLY | LIKE SLIGHTLY | LIKE MODERATELY | LIKE VERY MUCH | LIKE EXTREMELY
8 8 DISLIKE EXTREMELY | STRONGLY DISLIKE | MILDLY DISLIKE | NEITHER LIKE NOR DISLIKE | LIKE SLIGHTLY | LIKE MODERATELY | LIKE VERY MUCH | LIKE EXTREMELY
9 7 DISLIKE EXTREMELY | STRONGLY DISLIKE | MILDLY DISLIKE | MILDLY LIKE | LIKE FAIRLY WELL | LIKE QUITE A BIT | LIKE VERY MUCH

Figure 3. Scales investigated in the field surveys.

The following criteria were established to determine the relative adequacy of the scales: (a) ease of completion as shown by the amount of time required, (b) reliability as shown by the accuracy with which respondents duplicate results on an alternate form re-test, and (c) the amount of information obtained about the relative preference values of the group of foods.

(a) *Time required for completion.*

If there were major differences in the time required to complete the questionnaires, this would be an important criterion of relative efficiency of measurement. Proctors

recorded the time required by each respondent to complete the questionnaire in the 1952 survey, which included scales 1-5. These scales vary in length from 5 to 9 intervals. As expected, completion time is found to increase with the number of intervals; however, the difference between the shortest and longest median times is only 14 seconds. Obviously, this criterion needed no further consideration.

(b) *Reliability.*

Approximately 1250 subjects in the 1952 survey and all 1800 men in the 1953 survey were retested with a second questionnaire of the same scale type as the first. The retest took place as soon as all subjects had finished the first questionnaire. The same 20 food items appear on the second form, but are arranged in a different order. Product-moment correlations between responses to the same food items on these alternate forms are used to assess the reliabilities of the scale.

One striking result is the finding that reliability for certain food items is consistently high, whereas for others it is consistently lower. Differences among the reliability coefficients for various food items are much greater than differences among scales. To cite the extreme examples, the average correlation is +.92 for iced coffee, but only +.70 for jellied fruit salad.

TABLE 2

Averages[1] of test-retest reliability over 20 food items for nine scale types

Scale number	Number of intervals	Characteristics	First survey (1952)	Second survey (1953)
1	9	balanced, neutral	.821	.836
2	9	balanced, neutral	.833	
3	7	balanced, neutral	.848	
4	6	balanced, no neutral	.819	
5	5	balanced, neutral	.824	
6	9	balanced, neutral		.857
7	8	balanced, no neutral		.826
8	8	unbalanced, neutral		.814
9	7	unbalanced, no neutral		.826

[1] After transformation to Fisher's z statistic *(2)*.

Table 2 gives the reliabilities for the 9 scale types, obtained by averaging over all food items. They cover the restricted range from +.814 to +.857, and show no consistent relationship with the number of intervals on the scale. The differences among reliabilities of the scales are at a level for which statistical significance is doubtful; and certainly they are of little practical importance.

(c) *Transmitted information.*

The most meaningful criterion for assessing the relative values of scales is the amount of information transmitted *(3, 5)*. High transmitted information values indicate discriminating responses to the food items included in the survey, i.e., distinct and different distributions of responses for the various foods with a high level of agreement among the ratings for each. Since the ultimate objective is to have a scale as sensitive as possible to all differences among food preferences, the amount of transmitted information takes on great importance.

If other factors are held constant, the potential amount of information increases with the number of intervals. This follows from the nature of the information index, since, with more response intervals, there is greater opportunity for fine discriminations among stimuli. Several empirical studies have confirmed this relationship *(1)*, and it is borne out by results of the first survey. With one exception the information values increase as the number of intervals increases from five to nine. The 6-interval scale, No. 4, is an exception which led to the hypothesis that elimination of the midpoint, or neutral category, would increase the transmitted information. Results of the second survey tend to confirm this hypothesis.

Figure 4 is a graph of the transmitted information values obtained in both surveys, in which the scales are grouped according to number of intervals. Higher information values tend to go with the longer scale; however, the values associated with the two

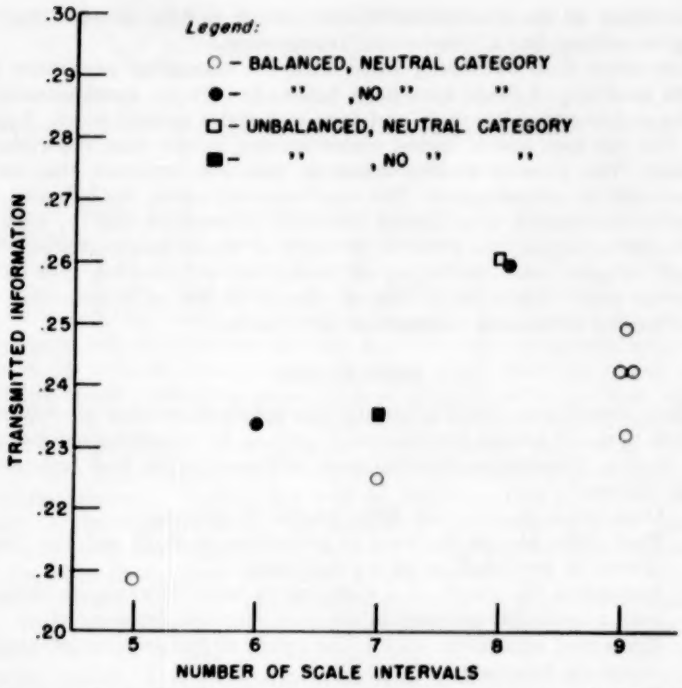

Figure 4. Transmitted information in relation to number of scale intervals.

8-interval scales are 5-10% higher than those for the 9-interval ones. This advantage appears both when the center category is omitted (No. 7), and when the *dislike moderately* category is omitted, leaving an unbalanced scale (No. 8). Even though the numerical differences among these indices are small, the consistency with which they appear is noteworthy. An appropriate non-parametric statistical test leads to rejection, at the .01 significance level, of the hypothesis that the differences are independent of number of scale intervals.

DISCUSSION

In one sense this research has failed to attain its objective, since no uniquely superior scale has as yet emerged. The similarities among the scale types investigated, particularly with regard to ease of completion and reliability, are more striking than the differences. The differences in transmitted information, although significant, are numerically small. However, the conclusion that it makes but little difference how a scale is constructed does not follow, because the range of scale types investigated was highly restricted. Selection and placement of the descriptive phrases on the basis of the vocabulary study was undoubtedly a most important factor; also, only those scale lengths were included which previous work has shown to be near the optimal range. No "poor" scales were specifically included as controls and the hedonic scale previously developed at the QM Food and Container Institute for the Armed Forces, Chicago, (No. 1) happened

to have many of the characteristics later shown to be desirable; thus the data give nothing like a "best-worst" comparison.

More often than not rating scales used for measuring preference and various qualities of foods have been balanced, with an equal number of positive and negative intervals, and have included a neutral point. Apparently this has been due to logical considerations, rather than experimental evidence. The present studies failed to find any evidence that either characteristic is advantageous. The two 8-interval scales, one balanced and the other unbalanced, gave almost identical information values; with the two 7-interval scales, the trend is in favor of the unbalanced scale. The neutral category was omitted in an 8-interval, a 7-interval, and in the 6-interval scale. Again, this omission caused no loss of information, but rather tended to increase transmitted information.

CONCLUSIONS

These results have implications for the practical problem of evaluating foods in terms of human preferences as well as for psychological measurement theory. Conclusions believed most pertinent to the food technologist are as follows:

a. Descriptive phrases may differ greatly in ambiguity.
b. They differ also in the level of preference implied, and this cannot always be predicted on an *a priori* basis.
c. Increasing the length of a scale, up to nine intervals, is related to only a negligible increase in the time required for completion.
d. Test-retest reliability, within the range of five to nine intervals, is relatively invariant.
e. Longer scales, up to nine intervals, tend to be more sensitive to differences among foods.
f. Elimination of the "neutral" category seems to be beneficial.
g. Balance, i.e., an equal number of positive and negative intervals, is not an essential feature of a rating scale.

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EFFECT OF CORN, BARLEY, STILBESTROL AND DEGREE OF FINISH UPON QUALITY OF BEEF

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On the Pacific coast there have been and still are instances where meat jobbers and fine eating establishments feature "eastern-fed beef"—that is, beef from east of the Rocky Mountain region of the U. S. A.—as their best stock in trade. The nature of the advertising together with the high reputation of the better restaurants naturally has caused the average customer to believe that corn-fed beef from the east is superior to that of other regions. There was a period, in years past, when the Pacific coast marketed almost exclusively grass-fed beef carrying more age than eastern beef. At that time the corn-fed beef produced in the midlands east of the Rockies was justifiably considered the most desirable in over-all quality. The picture has changed in recent years. The west is producing ever increasing amounts of grain-fed beef in feed-lots, and California ranks among the leading states in this regard. This change-over in western feeding methods has suggested an unbiased comparison of the organoleptic qualities of grain-fed beef with those of corn-fed beef.

Accumulated results of experimentation seem to have shown satisfactorily that subcutaneously implanted pellets of stilbestrol in beef animals increase daily gains and the feed efficiency of steers maintained on a fattening ration. This added factor in feeding methods, one that will be used more and more commonly by feed-lot operators, is also of interest with regard to its effect upon the eating quality of beef from animals so treated.

Another problem of investigative concern is the optimal degree of fatness in finished beef. Animal fats have been in surplus for some time and currently are selling below the "on-the-hoof" market prices of livestock. Incorporation of animal fats in feed rations is helping to lower the surplus, but it might be feasible and advantageous economically to decrease the rate of animal fat production by marketing animals with less finish. More investigation is needed, however, to determine at what stage of finish beef approaches its maximum eating quality. As Scott *(18)* has pointed out, the consumer is interested in tender, good-tasting beef that is not fat.

A number of investigators have reported on the effect of carcass fatness of beef on such organoleptic properties as tenderness, palatability and juiciness. Black *et al. (3)* found no significant differences in palatability of beef from steer carcasses ranging in fatness from about 26 to 31%. Earlier, Black, Warner and Wilson *(4)* reported little difference in palatability between beef rib samples containing 30% fat and those containing about 38%. Although the fatter ribs were somewhat more tender, these investigators concluded that "possibly 30% fat is sufficient to produce an aroma, flavor, and juiciness which compare closely with those of ribs possessing 38% fat." Callow *(5)* found palatability of beef roasts increased as the fat increased up to the point where fat made up about one-third of

the weight of the roast. When the fat made up a larger portion, palatability began to decrease. However, Barbella, Tannor, and Johnson *(2)*, in a study based on 728 animals of varying ages, weights, breeding, feeding, etc., in which the fat content of the edible portion of the 9-10-11 rib cuts ranged from 7.5 to 57.5%, demonstrated that fatness significantly enhanced the desirability of lean beef flavor. They also found a slight increase in desirability of the flavor of the fat with increasing fatness. The intensity of flavor of lean and fat showed no or negative correlations with fatness but seemed to depend most heavily upon age of the animal. Wanderstock and Miller *(19)*, comparing beef fed on pasture alone with beef given grain supplements and beef kept on dry lot, concluded that mainly because of increased fatness and higher carcass grade, the beef produced by feeding concentrates in addition to pasture, or in dry lot, was definitely superior to that produced by fattening in pasture alone. The principal differences in palatability observed were in factors of aroma, flavor of fat, flavor of lean, quality of juice and juiciness. In an investigation primarily concerned with the influence of an animal's age upon the quality and palatability of beef, Nelson, Lowe, and Helser *(15)* observed that in nearly every case fattened animals scored higher than unfinished ones. Flavor of lean meat was much more desirable when additional fat was imbedded within the muscle. However, for aroma and flavor of the fat itself the judging committee favored roasts without much finish.

The question of the influence of fatness of beef on juiciness has been exceedingly interesting. Juiciness has been estimated both by mechanical (press fluid) and organoleptic (panel scores) means, but most investigators have found no correlation, or at least no important correlation, between these measurements [Satorius and Child, *(17)*; Hardy and Noble, *(12)*; Gaddis, Hankins, and Hiner, *(9)*]. In a review of researches on the quality and palatability of meats, Coffey *(6)* reported that "the several methods developed for expressing juice from meat have brought out the fact that there is an unquestionable distinction between mechanical juiciness and palatability juiciness in cooked meat." Vacuum oven moisture determinations on samples of rib-eye muscle made in the course of work undertaken by Wanderstock and Miller *(19)* indicated that the fatter the carcass and the greater the degree of marbling, the lower the water content. This is in agreement with Gaddis, Hankins, and Hiner's *(9)* observation that with an increase in the percentage of fat in press fluid due to more intramuscular fat, the percentage of press fluid decreases. At the same time, judges' scores for quantity and quality of juice tended to increase. Their explanation for this seeming anomaly was that "fat adds flavor, resulting in stimulation of saliva, thus increasing the impression of juiciness, richness, and smoothness. It coats the mouth, causing a lasting impression of moistness during the chewing process." This may be the reason why in the work previously cited by Barbella, Tannor, and Johnson *(2)* the quantity of juice, measured by organoleptic scores, increased rapidly with fatness to 22.5% fat content, and then, more slowly, to 42.5% fat content; after that there was no apparent effect. They also observed that increased fatness gave a highly significant improvement in scores for quality of juice.

In attempts to correlate tenderness with degree of fatness there also seems to be some discrepancy between results obtained by panel judgments and results obtained by mechanical means. Hankins and Ellis *(10)* found little or no relation between fatness and tenderness in beef rib cuts as measured by a shear strength apparatus. On the other hand, Nelson, Lowe, and Helser *(15)* observed beef from feeders to be less tender than beef from similar cattle after fattening. However, as Ramsbottom, Strandine, and Koonz *(16)* pointed out, it is possible that changes in muscle fibers and connective tissue as well as fat content may have occurred during the fattening period. These investigators found no relationship between intramuscular fat and shear (measured by a Warner-Bratzler instrument) of raw or cooked muscle, though there were correlations between shear readings on cooked meat and shear readings on raw meat, organoleptic scores, and histological ratings. It is important to note, however, that their observations were made on a series of different muscles all from the same grade (U.S.D.A. *good*) of beef, rather than on the same muscle from a series of different grades of beef. Thus, the differences could have been due to differences in amount of connective tissue characteristic of the various muscles. Most investigators have concluded that while shear strength and similar objective tests measure satisfactorily a certain property of meat, these values are not related as closely as might be expected to tenderness as judged by a competent panel, [Deatherage and Garnatz *(7)*], and that no mechanical device is as convenient, reliable, and reproducible as panel testing for measuring tenderness of meat [Deatherage and Reiman *(8)*]. After studying 9 samples of beef muscle from each of 52 animals ranging from 10-week-old calves to 9-year-old cows, Hiner, Anderson, and Fellers *(13)* concluded that "tenderness in beef is a function of many interrelated factors, namely: breeding, feeding management, age, period of aging the raw meat, presence of collagenous and elastic fibers, size of fibers, the method of cooking the meat, and probably many others. Because of their interrelationships, an increase in our knowledge and control of any one of these factors alone will not necessarily result in tender meat."

EXPERIMENTAL PROCEDURE

In the present experiments an attempt was made to determine how much difference in eating quality was created by: 1) finishing steers on corn instead of barley; 2) feeding a higher proportion of concentrate to obtain a higher degree of finish—enough to achieve approximately U.S.D.A. *choice* grade as opposed to feeding to *good* grade*; and 3) implanting the animal with stilbestrol at the beginning of the feeding period, a procedure which tends to increase the amount of gain per pounds of feed. There were two trials, each involving 32 Hereford steers divided into 4 groups of 8 as shown in Table 1. All the animals came from the University of California's range herd and were above average in uniformity of breeding, age, and quality. The concentrate portion of the ration fed to the first 3 groups in both trials consisted of 65% ground barley, 20% molasses beet pulp, and 15% ground oats. Group IV in both trials received No. 2 yellow ground corn as concentrate. The hay fed to all groups consisted of 50-50 alfalfa and oat hay. Sixty milligrams of stilbestrol was implanted in the ear of each of the Group III steers in both trials.

* The actual grades which the animals made are noted on Table 1. Especially in the first trial, the actual distribution of *good* and *choice* grades was quite similar in the high and low concentrate groups.

TABLE 1

Animal history and feed-lot performance

	Trial 1 (Average days on feed—97)				Trial 2 (Average days on feed—185)			
	Group I	Group II	Group III	Group IV	Group I	Group II	Group III	Group IV
No. of steers	8	8	8	8	8	8	8	8
Initial age—mos.	27	27	27	27	20	20	20	20
Initial weight—lbs.	943	937	938	939	831	833	845	844
% concentrate in ration	24	54	50	53	22	59	61	59
Average daily gain—lbs.	1.8	2.4	3.2	2.6	1.8	2.4	2.7	2.2
Average daily feed consumption—lbs.	26.1	28.7	30.6	28.6	27.3	25.6	24.8	25.6
Feed per 100 lbs. grain—lbs.	1450	1196	956	1100	1476	1054	929	1138
U.S.D.A. slaughter grade:								
Prime—No. of animals	0	0	0	0	0	0	1	0
Choice—No. of animals	1	3	1	2	3	8	7	8
Good—No. of animals	6	5	7	6	4	0	0	0
Commercial—No. of animals	1	0	0	0	1	0	0	0
Average fat content of carcasses—%	33.3	35.6	33.0	36.4	34.6	42.6	41.8	40.1

At weekly intervals, 2 animals from each group were selected on the basis of comparable finish and slaughtered at a local packing house. After being graded by a U. S. Department of Agriculture inspector, the carcasses were allowed to age for 11 days before any subsequent analyses were made.

The fat content of the carcasses was estimated from specific gravity measurements of a 7-rib section (6th, 7th, 8th, 9th, 10th, 11th, and 12th ribs), made according to the procedure described by Hankins and Howe *(11)*. Lofgreen and Garrett *(14)* found a close relationship ($r = 0.93$) between the specific gravity and the fat content of the 9-10-11 rib cut as estimated by physical separation methods. This cut is closely related to the 7-rib section which has been found to be most representative of the beef carcass in composition by Hankins and Howe *(11)*. In the calculations of fat content (Table 1) the values 1.155 and 0.894, as reported by Lofgreen and Garrett *(14)*, were used as specific gravities of the fat-free body and beef fat, respectively.

Following the densiometric analysis, the 7-rib section was divided into 2 three-rib roasts (6th, 7th, 8th ribs and 9th, 10th, 11th ribs) for the organoleptic tests. The 12th rib was retained for further studies on composition.

A scoring method in which samples from each of the four groups were evaluated simultaneously was selected as the most efficient method for comparing the eating quality of the beef as affected by the various factors outlined above. It would also give data which could be used in obtaining correlation coefficients between the acceptance scores and any other desired quantitatively determinable properties of the meat. Using the designations of Table 1, the 4 groups of animals were:

I. Barley-fed, *good* grade
II. Barley-fed, *choice* grade
III. Barley-fed, *choice* grade, stilbestrol treated
IV. Corn-fed, *choice* grade.

Thus, the comparison between corn- and barley-fed beef was made on *choice* grade meat only, the influence of degree of finish (*good* vs. *choice*) involved only barley-fed animals, and the stilbestrol comparison was made on animals which had all been fed to *choice* grade on barley.

Both prime rib roasts and top round steaks were used for each of these comparisons. A relatively lean, less tender cut as well as a particularly tender part of the animal was therefore judged in each case. The weight of the rib roasts ranged between 7 and 11 lbs.; the top round cuts, between 3 and 4 lbs. Since 2 animals from each of the 4 groups were slaughtered each week of the experiment, the 4 sets of round steaks could be judged on 4 successive mornings and the corresponding rib roasts on the same afternoons. With 8 animals in each group, the experiment thus required 4 weeks, with a total of 16 judging sessions each on the 2 cuts from the 4 groups of animals. The experiment was initially carried out in May and June of 1954, and the entire design was replicated in January and February of 1955.

Roasting was selected as the method of cooking affording the best control and reproducibility. Both types of cuts were roasted, uncovered, in aluminum pans at 300°F. until the internal temperature as measured by a meat thermometer registered 150°F. For the rounds this required about one hour, and for the ribs, about 4 hours.

The meat was sliced approximately ¼ in. to ⅜ in. thick and cut in piece sizes of about 4 square inches. The pieces served each judge were matched as nearly as possible for location in the muscle, size, shape, and general appearance. Only the *longissimus dorsi* ("eye") and *spinalis dorsi* muscles of the rib roasts were used. Fat was trimmed off as completely as possible. The meat was served within 30 minutes of carving, the pieces being kept warm in their own juice on a steam bath in the interim. One piece from each of the 4 groups plus a duplicate of one sample, randomly chosen, was served each judge at each session. The duplicate sample permitted a check on the reproducibility and consistency of the judges' scores. The 5 pieces were placed in random order opposite code numbers on a warm plate. Judges were required to score each sample on a 7-point scale for tenderness, juiciness and flavor. A score of 7 indicated a *very desirable* sample with respect to that particular quality, 5 was *desirable* and 2 just *acceptable*. This scale is similar to that of the standard score sheet of the national project of the Cooperative Meat Investigations used by many research workers in this field [Alexander, Clark, and Howe *(1)*]. The judges were also asked to rank the samples from 1 to 5 in order of their "general preference."

The 14-member panel of judges was selected from a group of 23 interested staff members and students from various departments on the campus. None of the judges had experience in scientific tasting of meat, although some had participated in taste studies on other food commodities. Rib roasts and top round roasts of unknown origin representing the U.S.D.A. *choice, good, commercial* and *utility* grades were purchased at local markets and used in an extensive panel training program. The methods of preparation, serving and scoring the samples were analogous to those described for the experimental samples. The chosen panel of 14 was composed of judges who were most consistent in discriminating among the four grades. Other bases for selection included: range of scores used, performance on scoring duplicate samples, and genuine interest in the study. None of the judges appeared to be biased as to the geographical source of the beef.

RESULTS AND DISCUSSION

Table 1 summarizes the data on feed lot performance, slaughter grades, and fat content of the steers.

There was no difference in rate of gain, slaughter grade or fat content between Groups II and IV. Group III animals made significantly greater gains than those of any of the other 3 lots. In trial 1 the hormone-treated cattle had a significantly lower fat content than Groups II or IV. In trial 2, however, in which the feeding period was doubled, this difference was not observed.

The average scores and ranks for the 2 replications of the experiment are given in Table 2. The scores were subjected to an analysis of variance from which was derived the F-values and least significant differences (L.S.D.) at the 95% level of probability.

These results indicate that in the rib roasts there were few significant differences among the 4 groups in the quality factors judged. Among the rounds however, the *good* grade frequently scored lower than one or more of the three groups of *choice* animals. This was particularly noticeable in the flavor scores for both years, in the juiciness scores for the second replication (1955), and in the average ranks in the 1954 series. The differences were never very great, however, and probably would go unnoticed in a less carefully controlled situation. That is, if differences in the breed and environment of the cattle, differences in aging procedure, and differences in method of cooking were superimposed on the experimental variable of degree of finish (*good* vs. *choice*), as would normally be the case in practical situations, it is very unlikely that the *choice* meat would consistently be scored significantly higher than the *good*. The fact that a consumer ordinarily would not have the two grades of beef before him side by side for tasting at the same time is further reason to suppose that the small differences observed in this experiment would become negligible in more usual circumstances.

Average scores and ranks among the 3 groups of *choice* animals were quite uniform. Thus, it appears that from the standpoint of the organoleptic quality factors judged in this study, barley-fed beef is equal to that of corn-fed. From this particular study it is difficult to conclude that a real difference exists between the quality of meat from stilbestrol-implanted cattle and that of untreated animals fed a similar ration.

It is interesting that in the 1954 series, the corn-fed animals made very slightly better gains than the barley-fed; this was usually reflected in

TABLE 2
Average panel scores and ranks

Group	Ribs								Rounds							
	Tenderness		Juiciness		Flavor		Ranks		Tenderness		Juiciness		Flavor		Ranks	
	'54	'55	'54	'55	'54	'55	'54	'55	'54	'55	'54	'55	'54	'55	'54	'55
I	5.50	5.50	5.19	4.76	5.38	5.03	3.19	2.86	3.83	3.99	4.30	3.83	4.27	3.95	3.39	3.42
II	5.59	5.37	5.27	4.89	5.35	5.09	2.92	2.82	4.14	4.43	4.50	4.31	4.59	4.55	2.90	2.61
III	5.64	4.94	5.28	4.67	5.51	4.89	2.93	3.18	4.14	4.04	4.48	4.13	4.50	4.40	3.03	2.98
IV	5.34	5.10	5.37	4.74	5.45	4.97	2.95	3.13	4.33	4.04	4.63	4.11	4.58	4.38	2.69	3.01
F values	.92	2.48	.36	.21	.34	1.25	.87	.96	1.61	1.74	.41	2.14	1.84	7.61[2]	3.54[1]	3.30[1]
L.S.D. (95%)	.28	.34	.27	.27	.23	.15	.29	.38	.35	.31	.42	.28	.24	.20	.34	.47

[1] Significance between 95% and 99% (F-value greater than 2.95).
[2] Significance greater than 99% (F-value greater than 4.97).

TABLE 3

Correlation coefficients. Organoleptic scores vs. per cent fat

Groups I, II, III, and IV	Rounds		Ribs	
	1954	1955	1954	1955
Tenderness	.514 *	.289	.232	.379 [1]
Juiciness	.613 [2]	.046	.421	.197
Flavor	.389 [1]	.437 [1]	.107	−.162
Rank	−.447 [1]	−.568 [2]	−.428 [1]	.054
Groups I, II, and IV				
Tenderness	.665 [2]	.504 [1]	.235	−.269
Juiciness	.623 [2]	.243	.553 [2]	.188
Flavor	.516 [2]	.493 [1]	.320	.170
Rank	−.489 [1]	−.693 [2]	−.330	.015

[1] Significance between 95% and 99%.
[2] Significance greater than 99%.

slightly higher scores and slightly lower ranks for the corn-fed beef. In the 1955 experiment the reverse was true; the barley-fed group was generally rated better. The differences involved are very small, however, and the correlation may be entirely fortuitous.

Table 3 gives the correlation coefficients between the average scores and ranks for each of the 64 individual animals and its fat content calculated from the specific gravity of the meat. The degree of correlation is generally higher for rounds than for ribs, but none of the coefficients are especially high. Negative correlation between fat content and ranks is obtained, of course, since the preferred samples received the lowest average rank. When Group III (stilbestrol-treated) animals are eliminated from the calculation of correlation coefficients the correlations are slightly better. It appears, therefore, that the degree of finish, as reflected in the fatness of the beef, is not as important a factor as might be supposed in determining the flavor, tenderness, or juiciness of the meat.

SUMMARY

Organoleptic evaluation of rounds and rib roasts from four groups of steers (Group I, barley-fed to U.S.D.A. *good* grade; Group II, barley-fed to *choice* grade; Group III, barley-fed to *choice* grade and implanted with stilbestrol; Group IV, corn-fed to *choice* grade) revealed only minor quality differences in tenderness, juiciness, and flavor due to degree of finish, implantation with stilbestrol, or the particular grain used for fattening.

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DIFCO

Bacto-Dehydrated Media for Microbiological Assay of

VITAMINS and AMINO ACIDS

These media contain all the necessary nutriments for the growth of specified test organisms for the microbiological assay of vitamins and amino acids except for the component under assay. These basal media require only the addition of specified increasing amounts of the vitamin or amino acid being assayed to obtain a growth response which may be measured by acidimetric or turbidimetric methods for the construction of standard curves. The vitamin or amino acid content of the material under assay is determined by adding appropriate concentrations of the test substance to the basal medium and comparing the growth response obtained with that of the standard.

BACTO-RIBOFLAVIN ASSAY MEDIUM
BACTO-NIACIN ASSAY MEDIUM
BACTO-THIAMIN ASSAY MEDIUM
BACTO-PANTOTHENATE ASSAY MEDIUM
BACTO-B_{12} ASSAY MEDIUM USP
BACTO-CS VITAMIN B_{12} AGAR
BACTO-FOLIC ACID ASSAY MEDIUM
BACTO-PYRIDOXINE ASSAY MEDIUM
BACTO-BIOTIN ASSAY MEDIUM
BACTO-CHOLINE ASSAY MEDIUM
BACTO-CF ASSAY MEDIUM
BACTO-TRYPTOPHANE ASSAY MEDIUM
BACTO-LEUCINE ASSAY MEDIUM
BACTO-METHIONINE ASSAY MEDIUM
BACTO-LYSINE ASSAY MEDIUM
BACTO-ISOLEUCINE ASSAY MEDIUM
BACTO-ARGININE ASSAY MEDIUM
BACTO-TYROSINE ASSAY MEDIUM
BACTO-CYSTINE ASSAY MEDIUM

The method employed in carrying stock cultures of the test organisms and preparing the inoculum for microbiological assay is important. The following media have been developed especially for carrying stock cultures and for preparation of the inoculum:

BACTO-MICRO ASSAY CULTURE AGAR
BACTO-MICRO INOCULUM BROTH
BACTO-B_{12} CULTURE AGAR USP
BACTO-B_{12} INOCULUM BROTH USP
BACTO-NEUROSPORA CULTURE AGAR

BACTO-VITAMIN FREE CASAMINO ACIDS, dehydrated, is an acid hydrolysate of vitamin free casein prepared especially for laboratories investigating microbiological assay of vitamins.

Descriptive literature available upon request

DIFCO LABORATORIES

DETROIT 1, MICHIGAN